

Biology

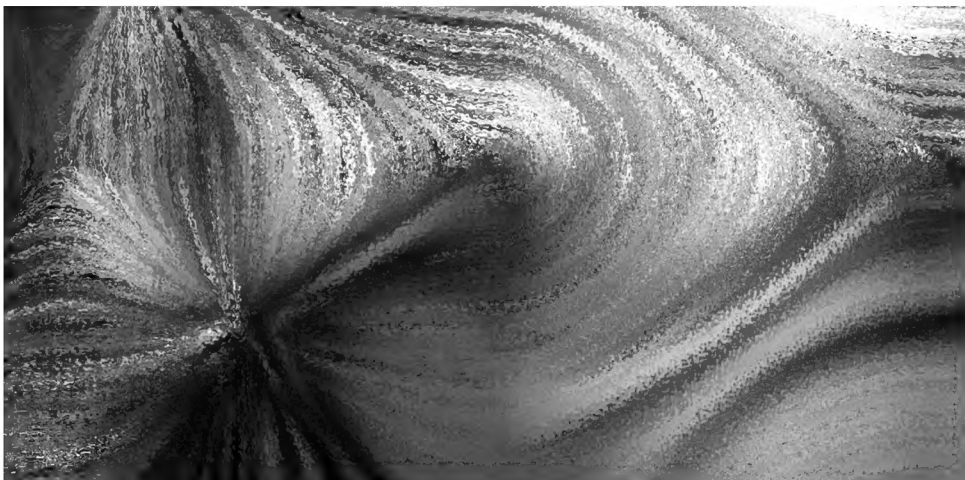
Teaching Resource

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Student Texts



Core Principles

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Core Principles

1.1

Biological Molecules

Content:

Carbohydrates

- ▼ The elements which make up carbohydrates. Monosaccharides as the basic unit from which other carbohydrates are composed: the reducing sugars glucose and fructose
- ▼ The condensation of glucose to form the disaccharide, maltose, and of glucose and fructose to form sucrose.
- ▼ The formation of the polysaccharides: starch, glycogen and cellulose
- ▼ Hydrolysis of disaccharides and polysaccharides
- ▼ The relationship of structure to function in starch, glycogen and cellulose

Proteins

- ▼ Amino acids as the monomers of which proteins are composed
- ▼ The condensation of amino acids to form dipeptides, polypeptides and proteins. Hydrolysis of proteins.
- ▼ The primary, secondary & tertiary structure of proteins. The relationship of structure to function in fibrous and globular proteins.

Lipids

- ▼ The elements which make up fats and oils
- ▼ Glycerol and fatty acids combine by condensation to produce triglycerides.
- ▼ Saturated and unsaturated fats
- ▼ The structure of phospholipids

Biochemical tests.

- ▼ The identification of reducing and non-reducing sugars, starch, proteins and lipids by means of simple biochemical tests, using Benedict's solution, iodine solution, the biuret test and the emulsion test.

Chromatography

- ▼ The separation & identification of compounds by means of chromatography, including R_f values and two way chromatography.

Water

- ▼ The biological importance of water as a solvent and as a medium for living organisms, including change of state and specific heat capacity.

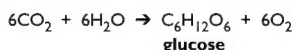
Introduction

It may surprise you to know that the bodies of living organisms are made up of fewer than 20 different chemical elements and just six of these bioelements make up 99% of the total body mass (oxygen 65%, carbon 18%, hydrogen 10%, nitrogen 3%, calcium 2%, phosphorus 1%). Most of the important biological molecules are organic, which means that they are made up from carbon compounds (note that most but not all compounds containing carbon are organic, exceptions are carbon dioxide and carbonates). Organic biomolecules include carbohydrates, proteins, lipids and nucleic acids, all of which exist as individual sub-units (**monomers**) or repeating chains of sub-units (**polymers**). Polymers are very large molecules, built up by **condensation reactions** in which a molecule of water is lost as each sub-unit joins the next. They may be broken down by the reverse process, **hydrolysis**, which involves the addition of a molecule of water for each bond broken. The following account describes the structure of carbohydrates, proteins and lipids and the relationship of molecular structure to function in living organisms.

CARBOHYDRATES

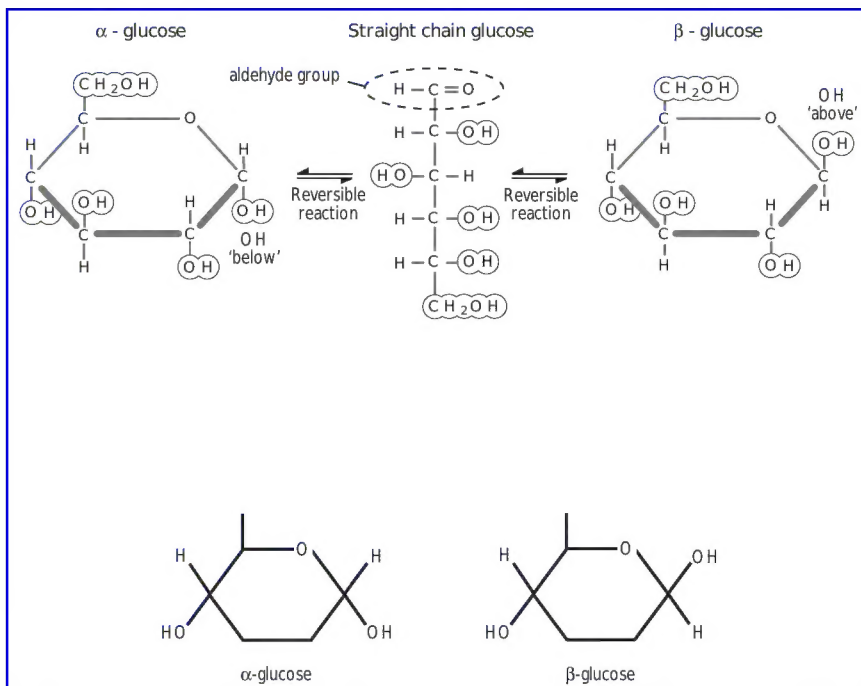
Monosaccharides: glucose and fructose

Carbohydrates are a family of molecules made up, as the name suggests, from carbon and the components of water (hydrogen and oxygen). Carbohydrates are manufactured by plants and are passed on to other organisms via food chains. You should already be familiar with the name and chemical formula of one of the simpler carbohydrate molecules, glucose, which is made from CO_2 and H_2O in the process of photosynthesis.



Glucose has six carbon atoms in the form of a framework to which the oxygen and hydrogen atoms are attached. In dry glucose powder the carbon atoms are arranged in a straight chain, but if it is dissolved in water, the chains form themselves into ring structures. Two different ring forms exist called **alpha glucose** and **beta glucose**. The carbon atoms are numbered 1 to 6 starting in a clockwise direction from the position of the oxygen atom. As discussed later, the different structures of alpha (α) and beta (β) glucose result in the formation of polysaccharides with different properties.

Alpha and beta glucose are **isomers**; that is they have the same chemical formula but a slightly different arrangement of atoms in the molecule. Fructose and galactose are also isomers of glucose. You will notice that fructose has the same number of carbon, hydrogen and oxygen atoms as glucose, but it has a keto group ($\text{C}=\text{O}$) instead of the aldehyde group (CHO). This gives fructose slightly different chemical properties, for example it is sweeter than glucose.



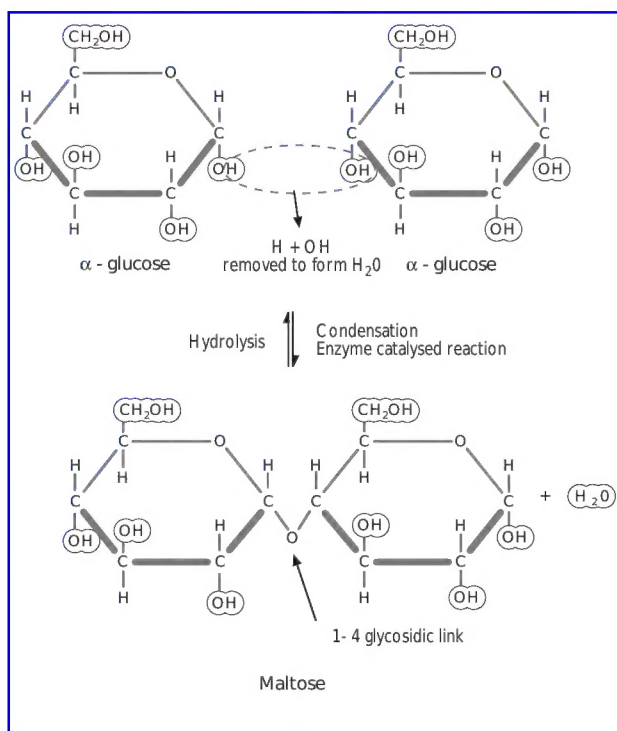
The condensation of monosaccharides to form disaccharides and polysaccharides

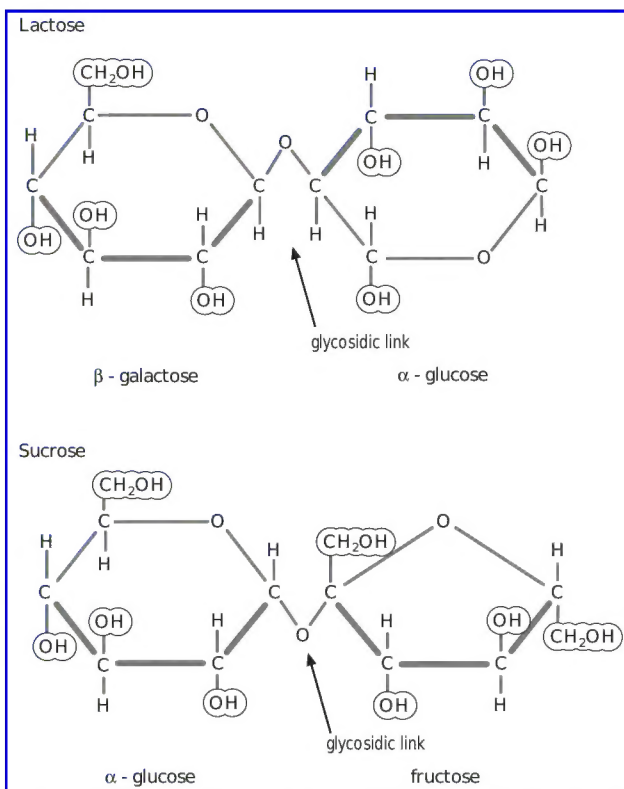
Ring structures like these are able to link together to form pairs or long chain molecules. The terms monosaccharide, disaccharide and polysaccharide are used to describe the number of glucose molecules making up a chain (mono = 1, di = 2, and poly = many). If the units of glucose link up to form larger molecules, the first carbon atom of one alpha glucose molecule links to the fourth carbon atom of another with the elimination of a molecule of water. This enzyme controlled reaction results in an oxygen bridge called a **glycosidic bond**. The formation of more than one bond by condensation leads to longer and longer 'multi-molecules' or polymers.

Disaccharides: maltose and sucrose

Maltose is a disaccharide formed from two molecules of alpha glucose, linked by an alpha 1-4 glycosidic bond. It is named after 'malt' which is germinated barley seeds used in the brewing industry. As the seeds germinate they activate amylase to hydrolyse their starch stores to maltose, upon which yeast acts in fermentation.

Sucrose is formed by a similar condensation reaction between a molecule of glucose and a molecule of fructose. Sucrose is the best known disaccharide as it the one used and known as 'sugar' in everyday life.



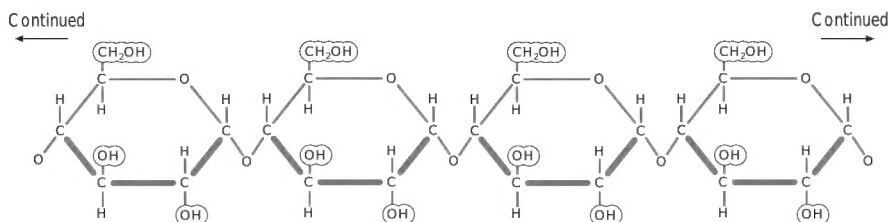


Polysaccharides: Starch, glycogen and cellulose (structure and function)

Glucose is the universal fuel for respiration, but it cannot be stored in cells because a high glucose concentration would result in the entry of large amounts of water by osmosis, which could cause cells unprotected by a cell wall or surrounding cells, to swell up and burst. For this reason, glucose in excess of energy requirements is converted for storage to the relatively insoluble polysaccharides **starch** in plant cells, and **glycogen** in animal cells. These substances are similar in chemical structure, both being formed from chains of alpha glucose molecules.

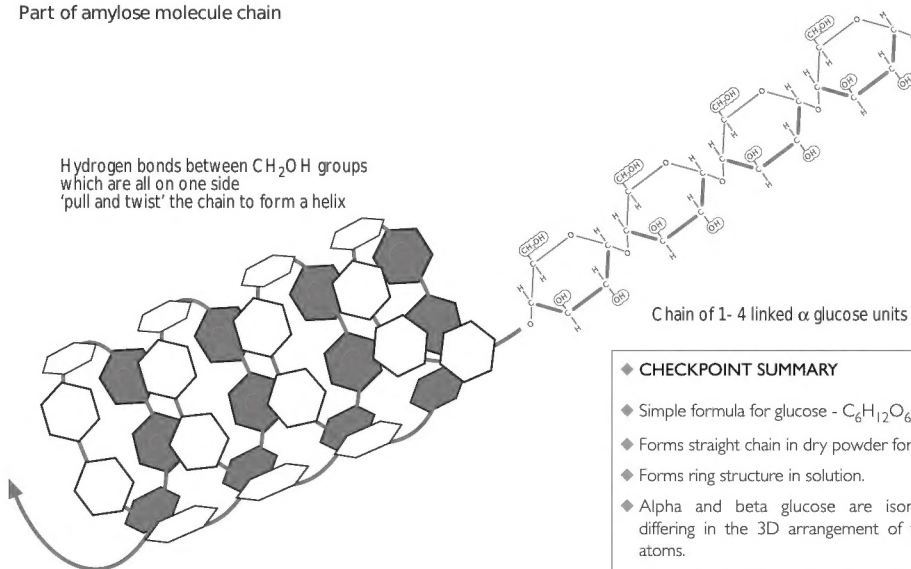
Starch is a mixture of **amylose** (unbranched chains of alpha glucose) and **amylopectin** (branched chains of alpha glucose). When combined together, the alpha glucose units have their oxygen bridges on the same side of the chain. Hydrogen bonds form between them and cause the chain to coil into a helix, so starch molecules have a dense, tightly packed structure and are relatively insoluble in water.

To dissolve starch, it is necessary to heat it. This breaks the hydrogen bonds and, as the coils begin to open, water molecules rush to occupy the exposed electrically charged (polar) sites, binding to them all over the surface. This is why starch suddenly swells in hot water. (Molecules which have charged regions are called **polar molecules**).



Part of amylose molecule chain

Hydrogen bonds between CH₂OH groups which are all on one side 'pull and twist' the chain to form a helix



Glycogen has a similar structure to amylopectin, but has a large number of shorter branches. It is more suited to animal metabolism as it is capable of being broken down more rapidly than starch.

Cellulose is the major component of plant cell walls. The characteristic fibrous structure of cellulose is created by chains of 1-4 linked beta glucose molecules. The beta molecules, when joined together, have their oxygen bridges on alternate sides of the chain, and thus it cannot form into a helix, but by forming hydrogen bonds with other chains lying side by side they make flat ribbons.

The basic meshwork of cellulose fibres in plant cell walls is strengthened and cemented by other materials such as pectin and lignin. Cellulose is the main component of numerous textiles e.g. cotton, linen, hemp; plastics e.g. celluloid and timber products; and paper.

Starch and cellulose have very different properties which arise as a result of one difference between alpha and beta glucose.

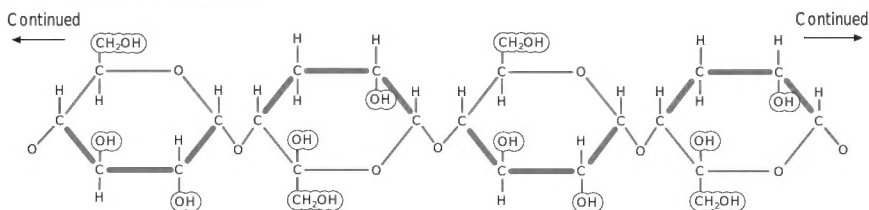
Starch has no distinct structure (amorphous), no structural strength, is slightly soluble in water, and digestible by vertebrates.

Cellulose has a distinct fibrous structure, great structural (tensile) strength, is insoluble in water and indigestible by vertebrates. (Herbivorous vertebrates harbour mutualistic microorganisms in specialised compartments of their gut which secrete cellulase enzyme which digests cellulose.)

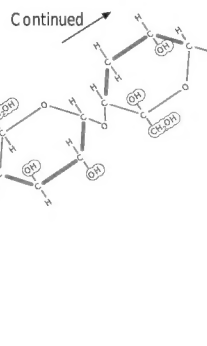
◆ CHECKPOINT SUMMARY

- ◆ Simple formula for glucose - $C_6H_{12}O_6$
- ◆ Forms straight chain in dry powder form.
- ◆ Forms ring structure in solution.
- ◆ Alpha and beta glucose are isomers differing in the 3D arrangement of their atoms.
- ◆ Monosaccharides (glucose, fructose and galactose) combine together with the elimination of water to form glycosidic bonds - a condensation reaction.
- ◆ Glycosidic bonds are broken by the addition of the elements of water across the bond (hydrolysis reaction).
- ◆ Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains).
- ◆ Both being long chains (polymers) of alpha glucose molecules.
- ◆ When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix.
- ◆ Starch molecules relatively insoluble in water.
- ◆ Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix.
- ◆ Instead they form hydrogen bonds with other adjacent chains to make flat ribbons.

Part of a cellulose molecule chain

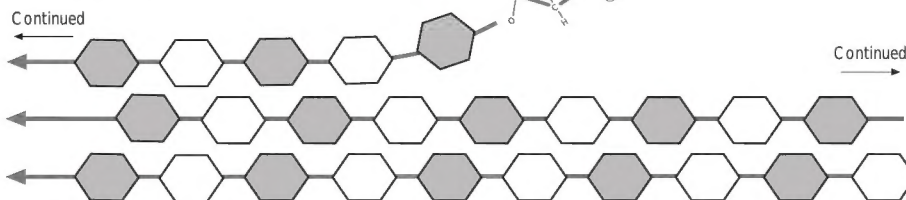


Notice how the CH_2OH groups alternate from side to side of the chain (the bonds indicated by a thick line are nearer)



Arrangement of molecules in microfibril

Chains of 1-4 linked β glucose units lie parallel forming a microfibril



Hydrogen bonds form between alternating CH_2OH units bind molecules together side by side into microfibrils.

Hydrolysis of disaccharides and polysaccharides

Mono, di and polysaccharides may be interconverted from one form to another by condensation, hydrolysis and isomerisation reactions, each particular reaction being catalysed in living cells by enzymes (see 10.5).

Most of the carbohydrate taken in by animals as food is in the form of polysaccharides or disaccharides. In the process of digestion, enzymes catalyse the hydrolysis of glycosidic bonds to produce monosaccharides which are then absorbed into the blood. A similar process occurs in the germination of seeds. Seeds store the polysaccharide starch which must be converted to glucose to fuel the energy needs for growth. The onset of germination is marked by the uptake of water, followed by the activation and release of hydrolysing enzymes.

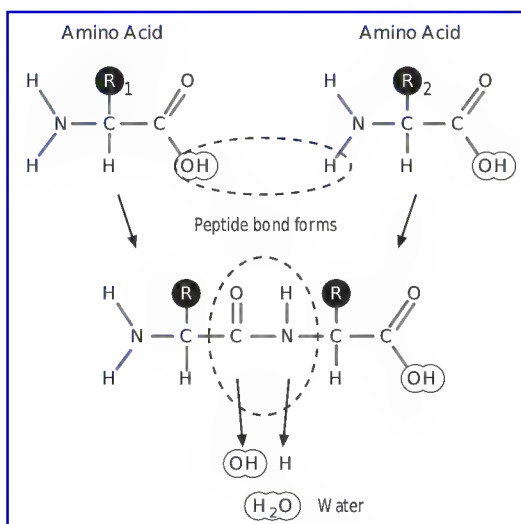
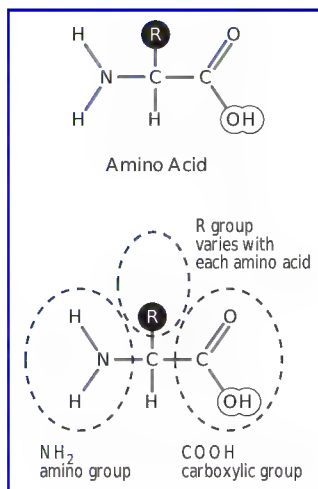
The hydrolysis of polysaccharides is achieved in two or three stages, each requiring a specific enzyme. This is illustrated by the commercial production of the glucose / fructose sweetener for soft drinks from low cost corn (maize) starch. Four enzymes are used in this process.

Proteins

Proteins are polymers made up of **amino acids**, joined together in long chains, which may be folded and twisted in various ways. There are as many as 100 000 different kinds of protein molecule in human cells. This variety of structure is made possible by the fact that the twenty different types of amino acids commonly found in proteins can be arranged in any order in chains up to 2000 units long.

Amino acids and peptide bonds

The chemical structure of an amino acid consists of a central carbon atom bonded to a hydrogen atom, a carboxyl group ($-\text{COOH}$), an amino group ($-\text{NH}_2$), and a side chain, which varies with each amino acid, referred to as the 'R' group. **Peptide bonds** form between the amino ($-\text{NH}_2$) and carboxyl ($-\text{COOH}$) groups of adjacent amino acids to make chains called peptides (dipeptide = 2 amino acids linked together, polypeptide = many amino acids). As in carbohydrates, these linkages are made by a condensation reaction involving the elimination of a molecule of water.



Primary structure

The amino acid sequence is the starting point or primary structure of proteins. The final shape of proteins depends upon additional bonds which form between the $-NH$, $-C=O$, and $-R$ groups which 'stick out' of the sides of the polypeptide chain.

Secondary structure

The secondary structure of proteins is determined by hydrogen bonds between adjacent $-NH$ and $-C=O$ groups. Two very different formations are possible, the **alpha helix** and **beta sheet**. Both of these secondary structures may occur in the same protein.

Tertiary structure

The tertiary (third level) structure of proteins is the complex 3D folding determined by bonds which form between R groups. There are three possible types depending upon the chemical properties of the different R groups. R groups may be acid, basic, polar or non polar.

Quaternary structure

The quaternary (fourth level) structure of proteins arises from the combination of separate polypeptide chains to form a larger complex, e.g. haemoglobin, which is composed of four polypeptide chains with iron containing haem units.

The relationship of tertiary structure to function

The types of bonding that occur in protein molecules determine their shape and their properties.

Hydrogen bonds are formed between polar $-R$ groups (in addition to the H-bonds already described)

Ionic bonds are formed between the negatively charged acidic $-R$ groups and positively charged basic $-R$ groups. The forces are like those which exist between the Cl^- and Na^+ ions in salt.

Hydrophobic bonds occur between the $-R$ groups of non polar amino acids e.g. valine (Val) and leucine (Leu).

Disulphide bridges are much stronger covalent bonds formed between the sulphur atoms of the amino acid cysteine (Cys).

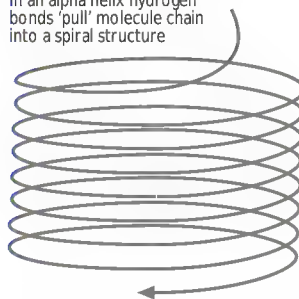
Proteins may be modified further by being linked to mineral ions, as in the case of haemoglobin or joined to carbohydrates and lipids to form **glycoproteins** and **lipoproteins**. This 'finishing off' process happens within the endoplasmic reticulum of cells.

Globular proteins

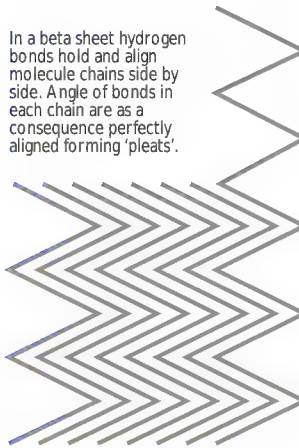
Globular proteins have a pronounced tertiary structure with many folds and twists, making them very susceptible to temperature and pH changes. Non polar groups tend to be repelled by water (hydrophobic) and tuck inside the molecule whilst the polar groups are attracted to water (hydrophilic) and they face outwards forming hydrogen bonds with water molecules. Globular proteins therefore

Secondary structure of proteins

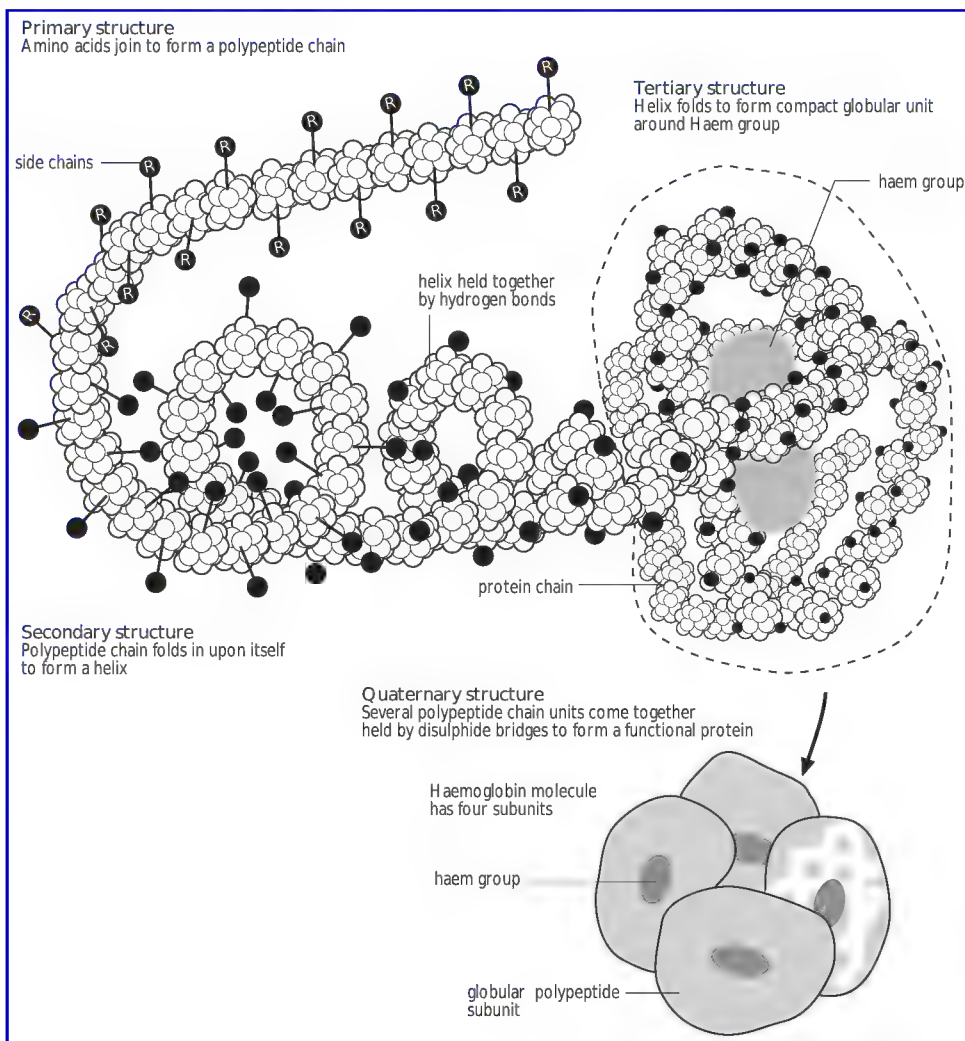
In an alpha helix hydrogen bonds 'pull' molecule chain into a spiral structure



In a beta sheet hydrogen bonds hold and align molecule chains side by side. Angle of bonds in each chain are as a consequence perfectly aligned forming 'pleats'.



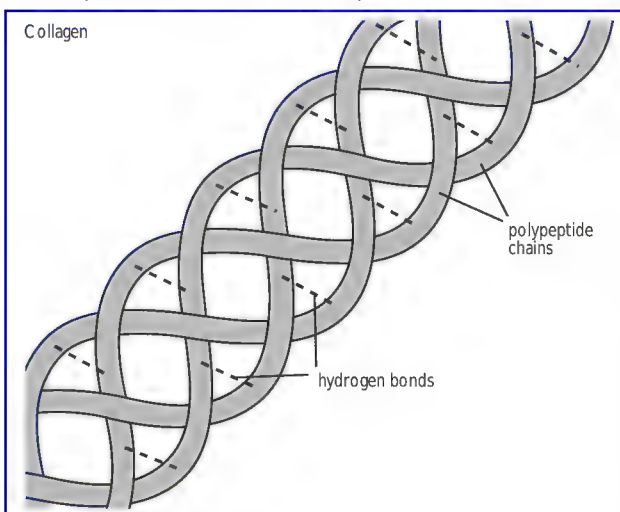
tend to be soluble. Globular proteins are involved in metabolic activities, and examples include enzymes, hormones, antibodies, and haemoglobin. **Haemoglobin** has a complex structure made up from four separate polypeptide chains (globins) joined to four iron (haem) ions. The iron (haem) groups in haemoglobin give the molecule its red colour and it is to these four sites that oxygen binds.



Fibrous proteins

In fibrous proteins the peptide chains are extended and inter-twined to form long thread like molecules. They do not dissolve in water and are relatively unaffected by changes in pH or temperature (they are not easily denatured). Fibrous proteins are mainly structural in function, giving support to tissues e.g. keratin in hair and skin, and collagen.

Collagen is the most important structural component of skin, bone and tendons. It is the collagen in skin which forms leather after treatment in the tanning process, and it is collagen which gives bones their 'bendability'. Bone without collagen is like concrete without the reinforcing steel rods. The word 'collagen' is Greek for 'glue maker' because it was extracted from bones by boiling them up in water and used as a strong wood glue. Chemically, collagen molecules are made up of three polypeptide chains, each consisting of about 1000 amino acid units which twist around each other like the strands of a rope (a triple helix). The molecules of collagen are insoluble and have a natural tendency to link up with each other forming fibrils several millimetres long.



Lipids

Lipids are a family of molecules which include fats, oils, waxes, phospholipids and steroids. Although they vary in chemical structure they share two important characteristics: they are energy rich substances, and they are insoluble in water.

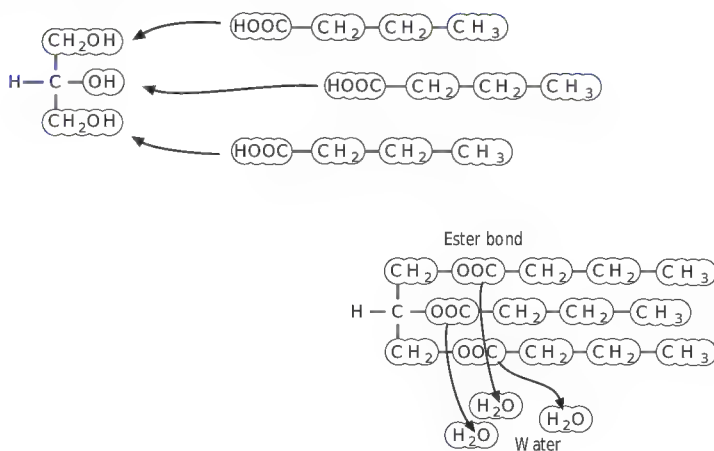
Structure and function of a triglyceride

Fats and oils are built on the same basic chemical plan which consists of a molecule of **glycerol** joined to three **fatty acid** chains. They are **triglycerides** ('mono' and 'diglycerides' are structures with one and two fatty acid chains respectively). The fatty acids are linked by **ester bonds** to glycerol in a condensation reaction similar to that seen in the formation of polysaccharides and polypeptides.

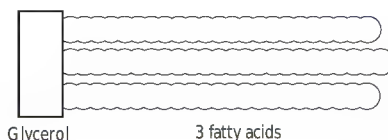
◆ CHECKPOINT SUMMARY

- ◆ All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH_2), a carboxyl acid group (COOH), and an 'R' group, all attached to a carbon atom.
- ◆ The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of $\text{CH}_2\text{NH}_2\text{COOH}$.
- ◆ Amino acids combine in a condensation reactions which form a peptide bond.
- ◆ There are about 20 different types of amino acids found in proteins of living organisms.
- ◆ Proteins are polymers of up to 2000 amino acids joined by peptide bonds (polypeptides)
- ◆ The combination of the 20 different types of amino acids gives rise to as many as 100 000 different types of proteins in living organisms.
- ◆ The sequence of amino acids in the polypeptide chain determines the primary structure of proteins.
- ◆ Hydrogen bonds between adjacent NH_2 and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein.
- ◆ Bonds that form between the 'R' groups determine the tertiary structure.
- ◆ Combination or association of two or more polypeptide chains determines the quaternary structure of a protein.
- ◆ Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.

Molecular model of development of a triglyceride

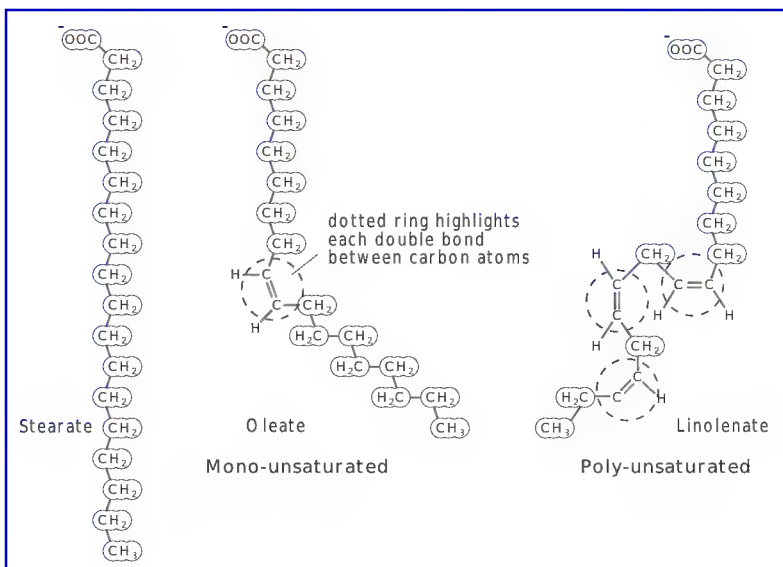


Model of a triglyceride



Fatty acids are hydrocarbon chains with a carboxylic (-COOH) group at one end. It is the -COOH group which forms the ester bond with glycerol. Hydrocarbons are non polar and, like fuel oils which have a similar structure, they contain a lot of energy locked up in their C-C and C-H bonds. Fats are mostly of animal origin and are solid at room temperature (RT); oils occur mostly in plants and are liquid at RT. The difference in melting point is due to the different nature of the hydrocarbon chains. In fats, each of the possible bonding sites between carbon and hydrogen is fully occupied (they are **saturated**), but in oils, one or more double bonds occur between the carbon atoms in the chain (they are **unsaturated**). Wherever a double bond occurs, the fatty acid chain acquires a kink, so the fatty acid chains of oils occupy more space than those of fats, giving them greater mobility and a lower melting point so that they are liquid at RT. The terms 'mono', 'di', and 'polyunsaturated' refer to the number of double bonds in the fatty acids.

When more food energy is taken in by the body than can be used, it may be converted to fat which is stored in large adipose cells under the skin and around internal organs. **Adipose** tissue not only acts as a long term food store; it also insulates the body against rapid changes in temperature, gives mechanical protection to internal organs and aids buoyancy in aquatic animals.

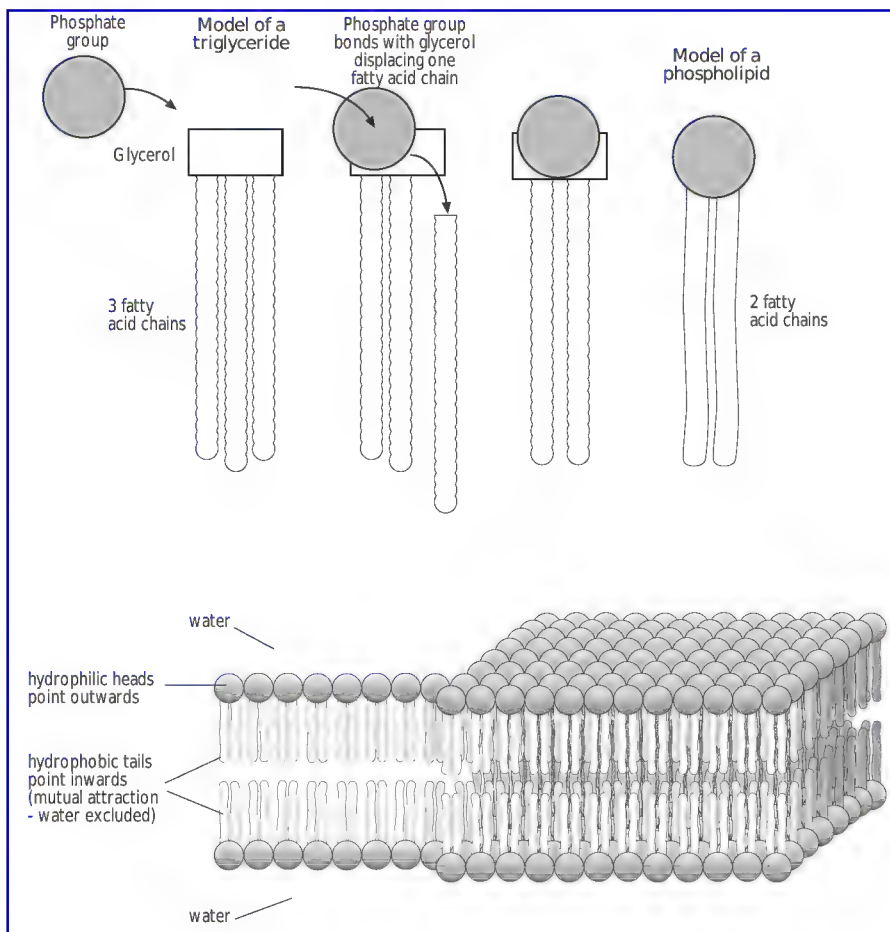


Waxes differ from fats and oils in that they have only one fatty acid chain and it is not joined to glycerol, but to a longer chain alcohol. They are produced in many animals and plants as a waterproof layer on external surfaces acting to reduce water loss (e.g. the cuticle of plant leaves, and the surface layer of some insect exoskeletons).

Structure and function of a phospholipid

Phospholipids are essential to cells because they form the structural basis of all membrane systems. They have two fatty acid chains (which may be saturated or unsaturated) joined to a molecule of glycerol, the third position being occupied by a phosphate group (phosphate plus choline or ethanolamine).

The phosphate group is strongly polar and readily dissolves in water, this is the **hydrophilic** (water loving) 'head' region of phospholipids. The non polar fatty acids are repelled by water and project in the opposite direction, and these are the **hydrophobic** (water hating) 'tails'. As a result, in the presence of water, phospholipid molecules form spontaneously into a characteristic **bilayer**. It is this bilayer which forms the molecular framework of all cell membranes.



◆ CHECKPOINT SUMMARY

- ◆ Triglycerides are composed of three (tri) fatty acids combined by condensation reactions to form ester bonds with glycerol.
- Fatty acids have long hydrocarbon chains and a carboxyl group (COOH).
- Saturated fatty acids (and hence saturated fats) have no double bonds in the hydrocarbon chain which is 'saturated' with hydrogen.
- Unsaturated fatty acids have one double bond.
- Polyunsaturated fatty acids have two or more double bonds.
- Lipids include fats, oils, waxes, phospholipids and steroids.
- Fats (triglycerides) are energy rich storage products in plants and animals.
- Phospholipids are formed from triglycerides by the replacement of one fatty acid by a phosphate group.
- Phospholipids form the structural basis of biological membranes by forming a bilayer.
- ◆ The bilayer is formed with their hydrophobic poles adjacent and their hydrophilic poles in contact with the aqueous phase.

Cholesterol is another important lipid component of cell membranes although it is very different in chemical structure to the lipids so far described. It has a ring structure and belongs to a group called **steroids** which include the sex hormones testosterone and oestrogen. Cholesterol molecules are strongly hydrophobic and they take refuge between the tails of phospholipids in membranes. Their presence in the bilayer has two effects. It slows down the lateral movement of individual phospholipid molecules, making the bilayer more stable, and it prevents the movement of certain substances through the bilayer, ensuring that membrane transport occurs through the correct (protein) channels.

BIOCHEMICAL TESTS

Test for reducing sugars e.g. glucose

All the common simple carbohydrate sugars, with the notable exception of sucrose (table sugar), have the ability to **reduce** other chemicals. They are thus called reducing sugars and they can be identified by heating with **Benedict's reagent**. Benedict's reagent contains copper sulphate and has a clear light blue colour. This changes to brick red as the copper is reduced to a solid precipitate of copper oxide in the presence of a reducing sugar. The quantity of reducing sugar in a positive test can be estimated from the amount of precipitate formed using a standard colour comparison sequence.

Test for non reducing sugars

Sucrose give a negative test with the Benedict's test, but when heated to boiling with dilute hydrochloric acid it is hydrolysed into its constituent monosaccharides e.g. glucose and fructose, which when made alkaline and re-tested with Benedict's reagent give a positive result.

If a mixture of both reducing and non-reducing sugars are present, which would be highly likely with plant tissue extracts, the precipitate from the initial positive Benedict's test would need to be removed by filtration, and the resultant clear filtrate re-tested for sucrose as described above.

Test for Starch

A solution of iodine in potassium iodide is added to the sample at room temperature. The presence of starch is indicated by a colour change from light brown to blue-black.

Test for Protein - the Biuret test

2cm³ of potassium hydroxide is added to 2 cm³ of the tissue extract in a test tube followed by a few drops of dilute copper sulphate solution. The presence of protein is indicated by a change in colour from light blue to purple.

Test for Lipids- the Ethanol Emulsion test

The sample is ground in ethanol to dissolve any fats and oils. 2 cm³ of the filtered extract is then added to 2 cm³ of water in a test tube at room temperature. The presence of lipids is indicated by the formation of a milky white emulsion.

◆ CHECKPOINT SUMMARY

- ◆ Generally all these tests are qualitative, i.e. the results show whether a substance is present or not, but they do not show how much
- ◆ They are not generally quantitative, i.e. the results do not give an accurate measure of how much of the substance is present.
- ◆ The Benedict's test for reducing sugars can be semi-quantitative in so much as the colour and amount of the precipitate found in a positive test is roughly proportional to the amount of reducing sugar present. Positive results can be compared to results from solutions of known concentration of reducing sugar.
- ◆ The same considerations apply to the iodine in potassium iodide test for starch. The colour for a positive test grading from blue-black to pale brown with decreasing amounts of starch present.
- ◆ The test for lipids is physical rather than chemical, depending on the observation of the formation of an emulsion.
- ◆ The biuret test is not a simple test for proteins but is used as such by Biologists
- ◆ These tests are commonly referred to as 'Food' tests as traditionally they are used on samples of food.

The technique of chromatography to separate and identify molecules

The separation of different substances in a mixed solution is mostly done by chromatography or electrophoresis. These techniques exploit the fact that different molecules and ions tend to move at different rates through a paper or gel medium.

Paper Chromatography

In this method, a concentrated sample of the mixture is formed by repeatedly placing small drops of it onto the same spot near one end of a piece of filter paper, letting the drop dry each time. The end of the paper is then dipped in a solvent solution such as ethanol and water. As the solvent front moves up the paper, so substances dissolved in it are carried along. The rate of movement for each substance depends upon its solubility in the solvent, so different substances move at different rates. The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a **chromatogram**, is allowed to dry.

By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_f (relative front) value can be obtained.

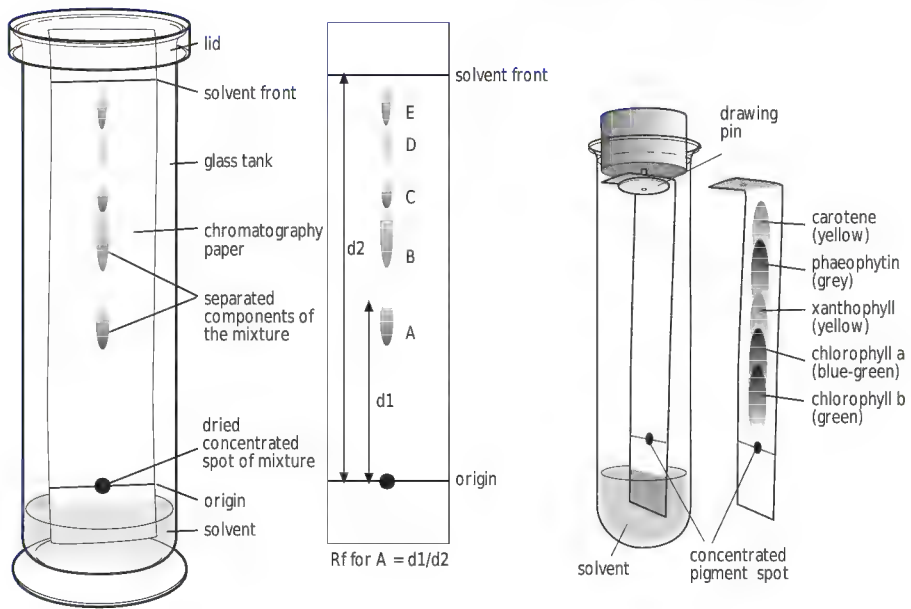
$$R_f = \frac{\text{distance moved by the a substance from its origin (d1)}}{\text{distance moved by the solvent front (d2)}}$$

The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a **two way chromatogram**.

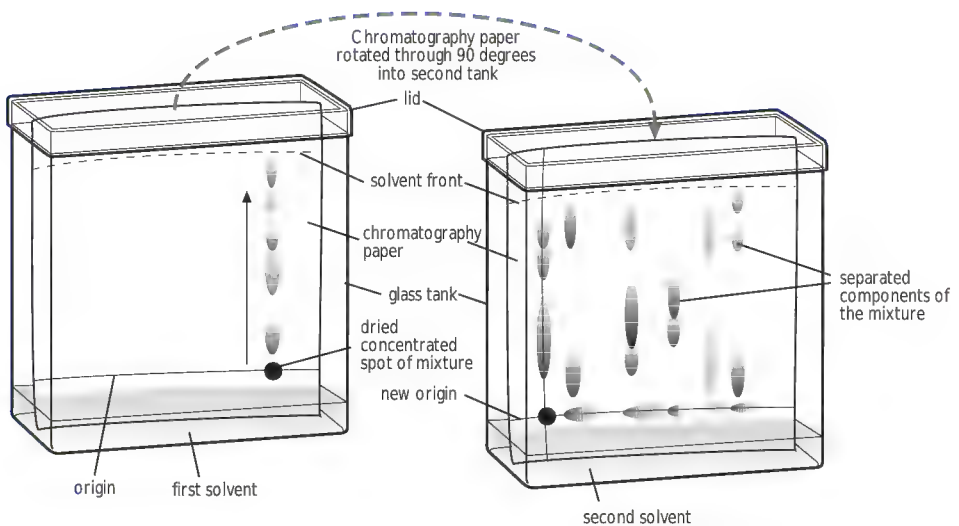
◆ CHECKPOINT SUMMARY

- ◆ Paper Chromatography is used to separate and identify small amounts of substances from mixtures
- ◆ In this method, a concentrated sample of the mixture is formed by repeatedly placing small drops of it onto the same spot near one end of a piece of filter paper; letting the drop dry each time
- ◆ The end of the paper is then dipped in a solvent solution such as ethanol and water. As the solvent front moves up the paper, so substances dissolved in it are carried along
- ◆ The rate of movement for each substance depends upon its different solubilities in the solvent and the water which is associated with the chromatography paper. The more soluble a substance is in the solvent the further it travels
- ◆ The paper is removed from the solvent before it reaches the top, then the solvent front is marked and the paper, now called a chromatogram, is allowed to dry
- ◆ By comparing the distance travelled by the solvent with the distance travelled by each component of the mixture, a comparative value, called the R_f (relative front) value can be obtained
- ◆ Distance moved by substance from its origin = d_1
Distance moved by the solvent front = d_2
Then $R_f = d_1/d_2$
- ◆ The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a two way chromatogram.

Separation of pigments in chlorophyll



Two way chromatography



WATER

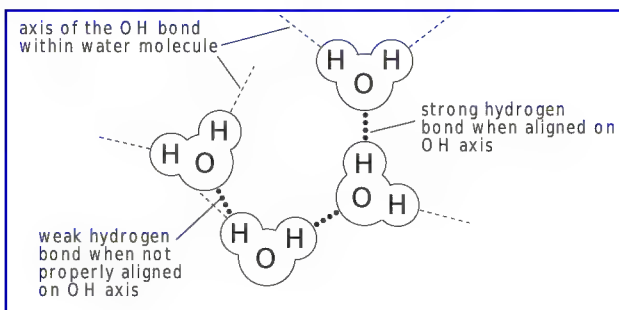
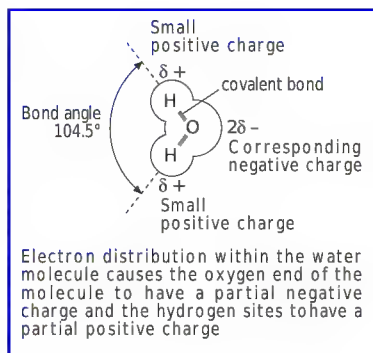
Almost everyone 'knows' the chemical formula for water - H_2O . It is a molecule made up of two hydrogen atoms combined with a single oxygen atom. As molecules go H_2O appears rather simple, yet its molecular structure results in many unique properties upon which life depends.

Each hydrogen atom combines with the oxygen atom by means of a pair of shared electrons (covalent bond). The nucleus of the oxygen atom has a strong positive charge which tends to pull the negatively charged electrons away from the smaller hydrogen atoms. As a result, a negative charge develops near the oxygen atom and positive charges occur near the hydrogen atoms. Molecules which have charged regions are called polar molecules. Water has both positive and negative charges and is therefore described as **dipolar**.

The positive and negative charges cause water molecules to attract each other by means of relatively loose linkages called **hydrogen bonds** (H-bonds). This mutual attraction explains why water is a liquid at standard temperatures and pressures (STP), whereas other, similar sized, non-polar molecules like methane (CH_4), ammonia (NH_3) and hydrogen sulphide (H_2S) are gases. The H-bonds in water are broken when energy is supplied, e.g. when water is heated. When they break the liquid becomes a gas (vapour). At and below 0°C the H-bonds are at their shortest and strongest and lined up to form a tight regular crystalline structure which is ice. Crucially for life on earth, ice is less dense or 'lighter' than liquid water; in other words ice floats, and aquatic organisms are protected beneath an insulating layer of surface ice. If it did not, and water froze from the bottom up, aquatic organisms would be exposed in progressively shallow surface waters.

Water molecules also attract other polar molecules. When mixed with salt (NaCl), for example, electrostatic links are made between the positive and negative charges of the salt and the water. Water is an excellent solvent. Any molecule with a polar region will dissolve, including sugars and alcohol, and water molecules gather around large protein molecules to form a special kind of solution called a **colloid**, as found in the cytoplasm of cells.

As you have seen, the chemical structure of water determines its properties. The table below lists these properties with examples of their biological importance.

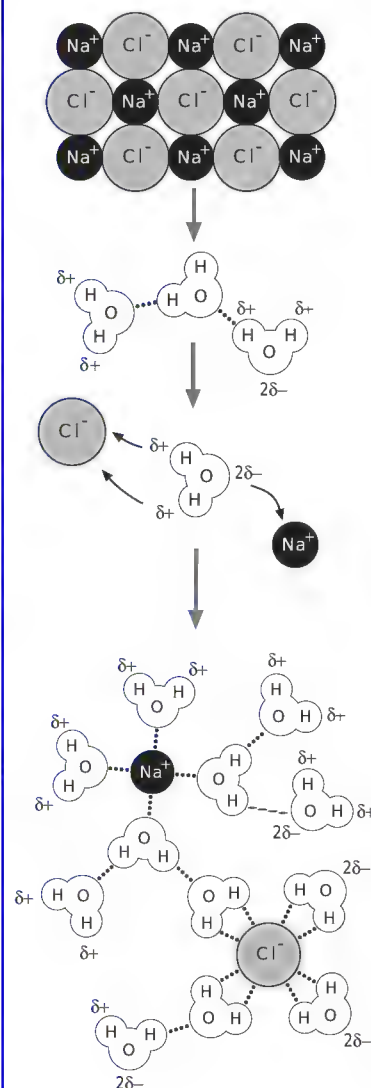


Property	Example of biological importance
State	Solid (ice) at 0°C and vapour above 100°C (at sea level), providing a wide range of temperature at which it exists as a liquid.
Solvent	'Universal' solvent in which many substances dissolve.
Density	Water supports aquatic organisms Ice is less dense than water; therefore ice floats and organisms can survive beneath it.
Viscosity	Allows free flow for transport of materials inside and outside of organisms.
Surface Tension	Water has a strong surface film allowing small organisms to be supported on top or underneath it
Capillarity	Water rises in small bore tubes against the pull of gravity because the polar water molecules are attracted to each other and to a number of surfaces. Capillarity is important in water transport in plants.
Incompressibility	Water provides a hydrostatic skeleton. When enclosed in any structure with a strong surrounding wall, water provides much support and protection to structures within it - e.g. earthworm body cavity, human abdominal cavity
Latent heat of evaporation	As water molecules evaporate they absorb heat energy from the surface they are evaporating from, cooling it down in the process, e.g. sweating.
Specific heat	Water has a high specific heat. This means that it gains and loses heat slowly which is important in the temperature control of aquatic environments and internal fluids.
Transparent	Allows the transmission of light e.g. for photosynthesis in aquatic organisms.

◆ CHECKPOINT SUMMARY

- ◆ Water molecules are dipolar with an electronegative pole and an electropositive pole
- ◆ Dipolar nature of water causes molecules to be attracted to each other by hydrogen bonds resulting in most of the key properties of water in relation to living organisms
- ◆ Liquid at normal temperature and pressures, solid (ice) at 0°C, boiling point 100°C, providing a wide range as a liquid
- ◆ It has high cohesion resulting in a strong surface film allowing small organisms to be supported on top or underneath it, and the ability for the water column in the xylem not to break under tension
- ◆ 'Universal' solvent in which many substances dissolve
- ◆ Water supports aquatic organisms, ice is less dense than water; therefore ice floats and organisms can survive beneath it.
- ◆ Low viscosity allows free flow for transport of materials inside and outside of organisms
- ◆ Water rises in small bore tubes by capillarity, important in water transport in plants
- ◆ Being incompressible water provides a hydrostatic skeleton, e.g. earthworm body cavity, human abdominal cavity
- ◆ Latent heat of evaporation results in a cooling process e.g. sweating
- ◆ Water has a high specific heat which is important in temperature control of aquatic environments and internal fluids.
- ◆ Transparency allows the transmission of light e.g. for photosynthesis.

Molecular model of water solubility



1.2

Cells

Contents

Cell structure

- ▼ The structure of an epithelial cell from the small intestine and a palisade mesophyll cell from a plant as seen with a light microscope. (see practical work)
- ▼ The ultrastructure of eukaryotic cells and their organelles (including functions)

Prokaryotic and Eukaryotic cells

- ▼ The ultrastructure of a typical bacterial cell
- ▼ Comparison of prokaryotic and eukaryotic cells

Electron microscopy and differential centrifugation

- ▼ The use of electron microscope and differential centrifugation as means of investigating cell structure and function.

Cell differentiation

- ▼ Tissues and organs

Introduction

All living organisms are composed of cells. It is calculated that an average human adult is made up of around 10^{14} cells, each coordinated with the others to form functional tissues, organs and systems. At the other extreme, some organisms are composed of a single cell; Amoeba, bacteria and yeast are examples of single celled organisms.

The word 'cell' to describe living units was first coined by the English Scientist Robert Hooke who, in the 1660s, used a simple microscope to observe the contents of a slice of cork from the bark of a tree. He noted that the structure resembled a multitude of tiny compartments like the cells inhabited by monks in a monastery - hence the term 'cell'.

Cells are grouped into two major types, **prokaryotic** and **eukaryotic**, the most important distinction between them being the presence or absence of a nucleus. The word '*karyon*' in Greek means 'nucleus', '*eu*' means true, and '*pro*' means 'before'. Eukaryotic cells are so named because they have a true nucleus and prokaryotic cells are built on an earlier design without a true nucleus.

Plant and animal cells as seen under the light microscope

Hooke used the word cell to mean a small compartment. When a slice of tissue from a plant or animal is viewed through a microscope, it is easy to see how this idea originated. Plant cells are generally easier to see under the microscope, as the cell membrane of each cell is surrounded by a relatively thick **cellulose cell wall** which gives each cell a more visible boundary.

The plant and animal cells illustrated here both show typical features as seen under a light microscope but both are specialised for their particular functions. The leaf **palisade cell** is located just beneath the upper epidermis of the leaf and it is specialised for the purpose of photosynthesis. It contains numerous chloroplasts which are aligned along the elongated vertical walls in such a way as to absorb the maximum amount of light. If living cells are observed it is possible to see the chloroplasts move within the cytoplasm to positions for maximum light absorption.

Columnar epithelial cells are found lining the small intestine. They are tall, relatively narrow cells with a nucleus towards the base, which are all attached to a basement membrane. They are specialised for the purpose of transport, and the membrane across which absorption occurs is adapted to provide a huge surface area by the possession of tiny extensions called **microvilli**.

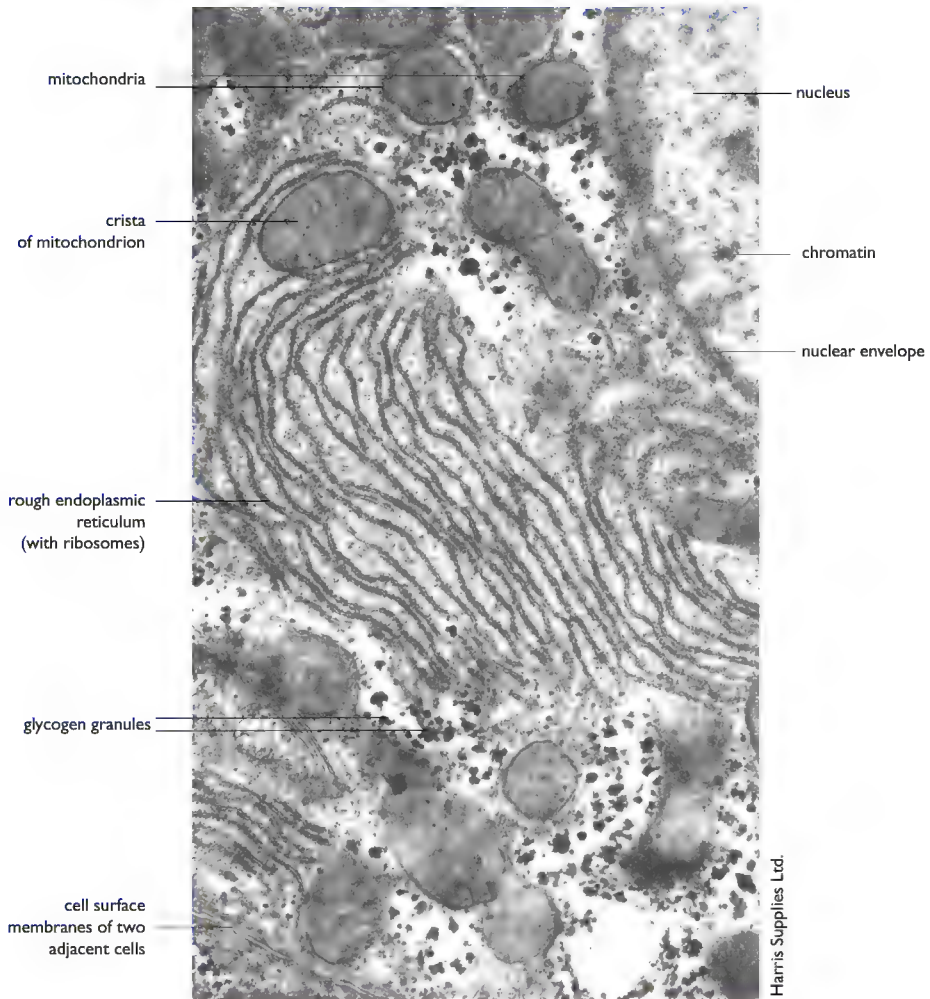
Table of differences between typical animal and plant cells seen under the light microscope.

Animal cells	Plant cells
Cell surface membrane	Cell surface membrane secretes cellulose cell wall
Small scattered vacuoles	Large central vacuole surrounded by membrane (tonoplast)
No chloroplasts	Chloroplasts with starch grains (in cells exposed to light)

◆ CHECKPOINT SUMMARY

- ◆ Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells.
- ◆ 'Typical' cells would be those considered to show features common to the vast majority of cells in animals or plants
- ◆ A mesophyll cell from a leaf could be considered as a 'typical' plant cell.
- ◆ Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body.
- ◆ In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplasts.

EM part of an animal cell (liver)

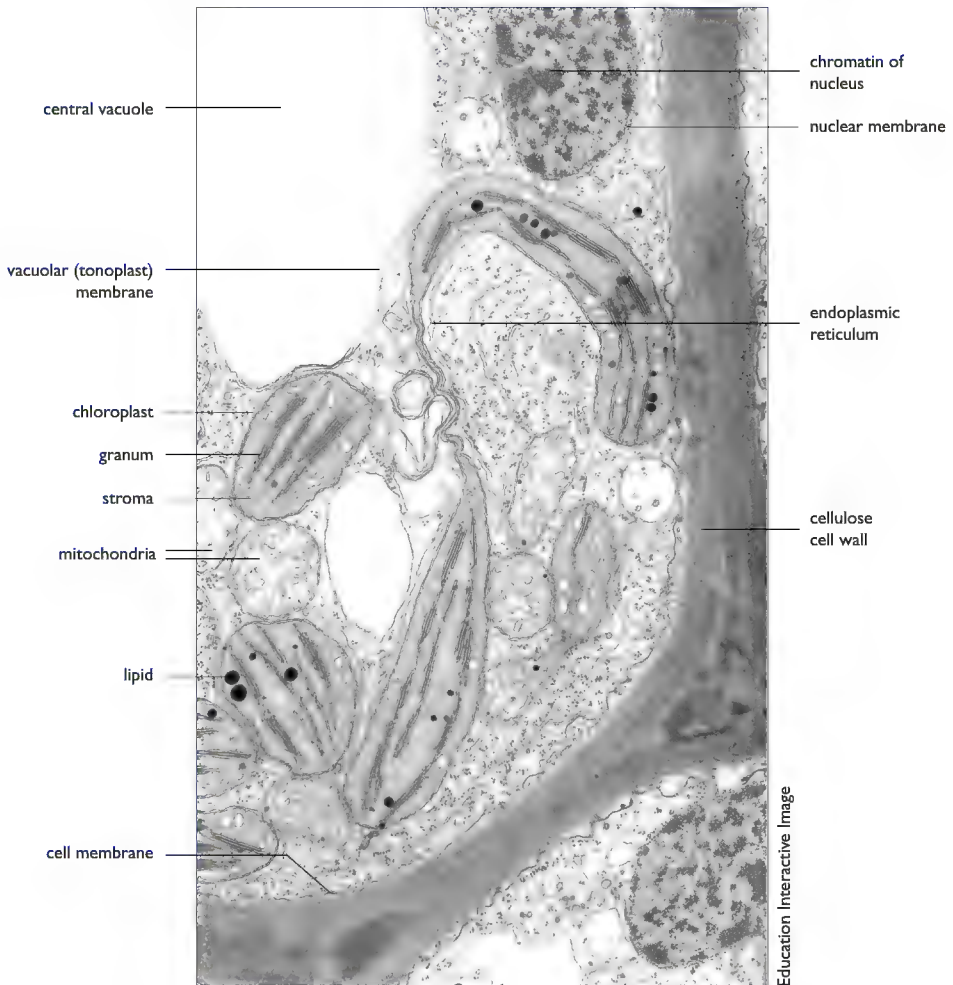


THE ULTRASTRUCTURE OF EUKARYOTIC CELLS

Nucleus - the Control Centre

Even with the optical microscope, the nucleus is clearly visible, having an average diameter in animal cells (liver) of about $5\text{ }\mu\text{m}$, and in plant cells (mesophyll) of about $15\text{ }\mu\text{m}$. The nucleus stands out when cells are viewed under the microscope because of the material **chromatin**, a mixture of DNA and protein, which takes up the stains used in slide preparations. Chromatin is the material which forms the chromosomes just before nuclear division. The nucleus is surrounded by a double membrane, the **nuclear envelope** which has the same structure as, and is continuous with, the other

EM part of plant leaf cell



membrane systems of the cell. Three or four thousand pores (up to 100nm diameter) perforate the nuclear envelope but the passage of materials in and out is controlled by plugs of protein and RNA which appear to fill the pores.

Genes in the nuclear chromosomes direct all the cell's activities and determine its life span and reproductive cycle. The nucleus is rightly called the command centre, but it does not contain all of the genetic material of the cell. Mitochondria and chloroplasts also contain DNA and have genes of their own.

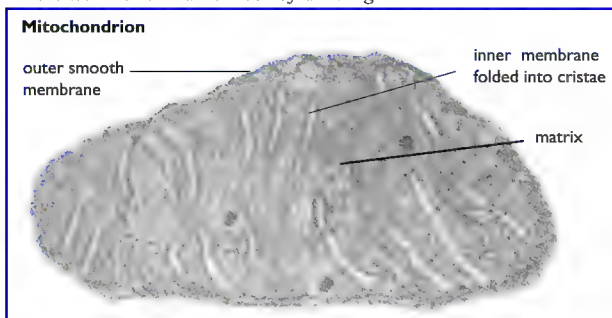
Nucleolus

This is an even more darkly stained region in the nucleus, which consists of a mass of RNA, used in the manufacture of **ribosomes**. Ribosomes are relatively small structures, about 20 nm in diameter which occur in large numbers, often several thousand per cell either bound to internal cell membranes or free in the cytoplasm. They are built in two sections called sub-units, made of approximately equal quantities of RNA and protein. They act as the sites of assembly for new proteins, manufactured both for export and for internal use.

Mitochondria - Centres of aerobic respiration

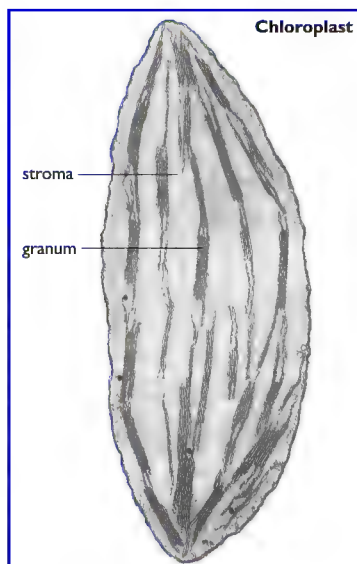
Mitochondria (singular = mitochondrion) carry out aerobic respiration, which transfers energy from energy rich substances such as glucose, into the energy rich substance ATP. High energy consuming tissues like those in the muscles or brain may contain up to a thousand mitochondria in a single cell.

Mitochondria can vary greatly in length and shape, but for reasons of surface area to volume ratio, they are never greater than 1µm in diameter. There is an outer and an inner membrane. The area of the inner membrane is made as large as possible by infoldings of the membrane (**cristae**), and it is encrusted on its inner surface with spherical bodies which contain the enzymes for making ATP. The more active the cell, the more cristae and the more enzymes there are. In fact the more active the cell, the more the mitochondria increase in size and number by dividing.



Chloroplasts - Synthesis of organic materials

Chloroplasts contain the green pigment chlorophyll which absorbs light for photosynthesis. In photosynthesis light energy is used in the synthesis of organic compounds (e.g. glucose) from carbon dioxide and water. The energy is trapped in a stable form in the organic compounds. Chloroplasts, like mitochondria, have a double membrane, but there is also a third membrane system inside the chloroplast called the **thylakoid** membrane system. It is on this that the chlorophyll molecules are located. The thylakoid membrane system is arranged in the form of stacked discs (**grana**) joined by connecting membranes (**lamellae**). The internal space is filled with a fluid (**stroma**) containing enzymes for making organic compounds, e.g. glucose and starch.



Plasma (cell surface) membrane

This controls the entry and exit of all materials into and out of all cells. The electron microscope does not reveal any details of its structure, which is modelled on evidence of biochemical investigations. It is fatty in nature and contains proteins and other substances. It is partially permeable, being more permeable to water, than to dissolved solutes which pass through at different rates depending on a complex of factors.

In cells which are specialised for the exchange of materials across the plasma membrane e.g. cells lining the small intestine and parts of the kidney tubules, the surface area may be dramatically increased by microvilli. Microvilli are extensions of the plasma membrane which protrude out of the surface like the bristles of a hair brush.

Rough and smooth endoplasmic reticulum

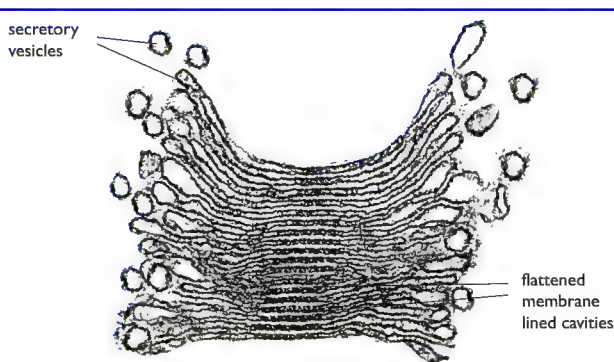
In addition to the outer surface (plasma) membrane and those which surround the organelles, cells (especially secretory cells), have extensive internal membrane systems, known as the endoplasmic reticulum (ER). The **ER** is a maze of membrane bound tubules and layers of flattened sacs enclosing a fluid filled interior, which acts as an intracellular (inside the cell) transport and storage system. It is continuous with the nuclear membrane and similar in structure.

The rough ER has ribosomes associated with it on the cytoplasmic side of the membranes which are the sites of protein synthesis.

The smooth ER tends to be more tubular, and appears to have a complex set of functions, including fat metabolism.

Golgi apparatus

This is more clearly seen in animal cells and is a region of stacked smooth ER specialised for the purpose of collecting, processing and redirecting proteins and fats made in the rough ER. Materials are transported between the ER, the Golgi apparatus, and the plasma membrane, sealed in membrane sacs called vesicles which bud off from one membrane, and then fuse with another. Substances are taken in (**endocytosis**) and expelled (**exocytosis**) by the plasma membrane in this way. For example, digestive enzymes produced by cells of the pancreas are secreted by exocytosis into the pancreatic duct.



Lysosomes

Vesicles produced by the Golgi apparatus are specialised for particular functions. Animal cells, have lysosomes which contain a cocktail of digesting enzymes. They break down and dispose of unwanted materials or damaged structures in the cell, expelling waste debris by exocytosis, and recycling reusable molecules within the cell. They also meet and fuse with vesicles carrying imported food substances, or immobilised bacteria, from the cell surface, and digest the contents.

It is clear from this that the membranes surrounding lysosomes must act as impermeable barriers preventing the leakage of its digestive enzymes into the cytoplasm, this process (**autodigestion**) occurs after the death of a cell and plays an important part in its removal.

Cell wall

The plant cell wall is made of a fibrous cellulose framework mixed with other materials, notably **pectin**. Pectin is a glue like material (it is used in jam making to set the jelly) and it is concentrated on the surface, sticking adjacent cells together. This sticky region can be seen as a line on electron micrographs and is called the middle lamella. When stretched around an expanded (turgid) cell, the cell wall makes a considerable contribution to the support of non-woody structures, e.g. leaves. The cell wall is fully permeable to water and dissolved solutes, but influences the exchange of these across the selectively permeable cell membrane, by exerting various physical and chemical forces. The wall is perforated by tiny holes, through which strands of cytoplasm (**plasmodesmata**) pass giving direct communication between neighbouring cells.

In 'woody' parts the cell wall is impregnated with **lignin**. Lignin is a hard, impermeable substance, and lignification typically results in the death of the cell. Such dead lignified tissues are specialised for water transport (e.g. xylem) and support (e.g. xylem and sclerenchyma fibres).

◆ CHECKPOINT SUMMARY

- ◆ The plant cell wall is made of a fibrous cellulose framework mixed with other materials, notably pectin sticking adjacent cells together as the middle lamella
- ◆ When stretched around an expanded (turgid) cell, the cell wall makes a considerable contribution to the support of non-woody structures, e.g. leaves. In 'woody' parts the cell wall is impregnated with lignin
- ◆ Plasma (cell surface) membrane controls the entry and exit of all materials into and out of all cells. It is fatty in nature and contains proteins and other substances. It is partially permeable, being more permeable to water, than to dissolved solutes
- ◆ The nucleus is surrounded by a double membrane, the nuclear envelope. Genes in the nuclear chromosomes direct all the cell's activities and determine its life span and reproductive cycle. The chromosomes are only formed just before nuclear and cell division
- ◆ Mitochondria (singular = mitochondrion) carry out aerobic respiration, which transfers energy from energy rich substances such as glucose, into the energy rich substance ATP. High energy consuming tissues like those in the muscles or brain may contain up to a thousand mitochondria in a single cell
- ◆ Chloroplasts are the centres of photosynthesis. They contain the green pigment chlorophyll which absorbs light for photosynthesis in which light energy is used in the synthesis of organic compounds (e.g. glucose) from carbon dioxide and water
- ◆ Rough endoplasmic reticulum is a maze of membrane bound tubules and layers of flattened sacs enclosing a fluid filled interior, which acts as transport and storage system within the cell. It has ribosomes associated with it which are the sites of protein synthesis
- ◆ The smooth ER tends to be more tubular, and appears to have a complex set of functions, including fat metabolism
- ◆ Golgi body is more clearly seen in animal cells and collects, processes and redirects proteins and fats made in the rough ER. Materials are transported between the ER, the Golgi apparatus, and the plasma membrane, sealed in membrane sacs called vesicles.

Prokaryotic and eukaryotic cells

The plant and animal cells which have been described so far have complex membranes systems, membrane-bound organelles, and their genetic material is enclosed within a double nuclear membrane. They are called eukaryotic cells ('eu' means 'true', and 'karyon' is Greek for 'nucleus'). The first organisms to develop on earth were of a simpler design similar to that of modern day bacteria. They are unicellular, much smaller than eukaryotic cells, usually not more than $1\mu\text{m}$ wide and their DNA is not enclosed within a nuclear membrane. They are called prokaryotes ('pro' means 'before').

Bacterial cell structure of a generalised type is illustrated here in diagrammatic form. Compare and contrast this structure with that of the eukaryotic cells. Most obviously, there are no large membrane-bound organelles. Instead of mitochondria and chloroplasts, the enzymes for respiration (and photosynthesis where this occurs) are located on simple membrane systems. In the absence of a nucleus, the DNA is formed, with its associated proteins into a single main coil, the bacterial chromosome. Additional small loops of DNA called plasmids may also occur. Not all prokaryotes are able to move, but some possess flagella, which are like elongated cilia but without the microtubules, capable of propelling the organism through water. The cell wall is not made of cellulose, but is formed from a mixture of polysaccharides and amino acids called murein. Many bacteria which cause disease (pathogenic bacteria) have a slime capsule as their outermost layer which helps in protection against the body defences of the infected organism.

The diagram is generalised to show all the possible features that might occur in a prokaryotic cell. It does not represent a real organism. Prokaryotic cells generally divide and reproduce themselves much quicker than eukaryotic cells. When grown in ideal conditions, *Escherichia coli* can grow and divide every 20 minutes, so a single cell could theoretically give rise to 2^{36} (that is a 2 with 36 noughts) in just 12 hours!

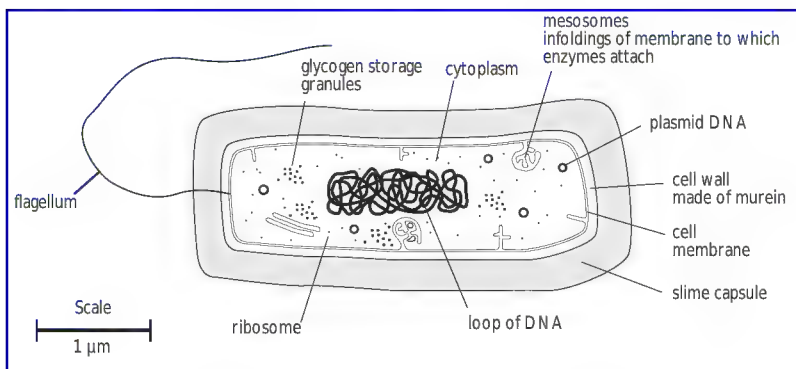


Table of principle differences between prokaryotic and eukaryotic cells

Prokaryotic cells		Eukaryotic cells
Size	smaller (1-10 μm)	larger (10-100 μm)
Nucleus	no nucleus	true nucleus bounded by a double membrane (nuclear envelope).
DNA /	DNA occurs in a loop	DNA attached to chromosome histone proteins forms separate chromosomes
Organelles	simple infoldings of membrane only	mitochondria, chloroplasts, centrioles, microtubules may all be present ER present with Golgi apparatus and associated vesicles and lysosomes
Cell walls	always present and made of murein	In plants and fungi only, and made of cellulose (plants) or chitin (fungi)

◆ CHECKPOINT SUMMARY

- ◆ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles
- ◆ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm
- ◆ The cell wall is not cellulose as in plant cells
- ◆ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria
- ◆ Ribosomes present in both prokaryotes and eukaryotes
- ◆ Prokaryotic cells may form colonies but never multicellular organisms/tissues
- ◆ Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously
- ◆ Eukaryotic cells typically form tissues in multicellular organisms.

Electron microscopy

For the visual study of cell structure it is necessary to enlarge (magnify) them by the use of microscopes. The **light microscopes** that you are most likely to come across in general Biology laboratories usually magnify up to 400 times (special techniques could take this up to 1000 times). However, to continue magnifying objects past a certain point reveals no further detail. In order to see more detail it is necessary to upgrade the resolving power (resolution). **Resolution** or resolving power can be defined as the ability of an optical system (e.g. microscope, the eye) to distinguish (resolve) detail in an image of an object.

The amount of detail that can be seen in an image is determined by the resolving power of the microscope system being used. In the case of looking at material under the light microscope the resolving power depends upon the light being able to distinguish this detail. To do this light must be reflected back from structures. Any structure less than 0.2 micrometres (μm), that is 200 nanometres (nm) in diameter cannot be distinguished by light, nor can two structures closer than 0.2 μm be seen as separate.

(1 μm = one thousandth of a millimetre, 1 nm = one millionth of a millimetre)

To improve the resolution, it is necessary to use a device which uses a beam of electrons, instead of light, called the **electron microscope**. Electron beams have a much shorter wavelength than light and give a hundred times better resolution. The magnification of an electron microscope picture (electron micrograph) can also be increased significantly to reveal this extra detail. The most powerful electron microscope can magnify up to x 500 000.

There are two types of electron microscope. In the **transmission electron microscope (TEM)**, an extremely thin section of the specimen is held on a copper or nickel grid and an electron beam passes right through it. Inside the electron microscope tube, electrons are accelerated by high voltage and focussed into a fine beam by a strong electromagnet (condenser lens). The beam passes through the specimen and is focussed further by additional electromagnets, referred to as the objective, intermediate and projector lenses, to give an image on a fluorescent screen, similar to the screen of a television set.

Electrons are disrupted by particles in the specimen to produce lighter and darker areas on the screen which correspond to dense and less dense parts of the specimen. The higher the density, the greater the scattering and the lighter the resulting picture on the fluorescent screen. If a photographic film plate is substituted for the fluorescent screen, the resulting picture is called an **electron micrograph**. A vacuum is maintained inside the tube of the electron microscope so that the electron beam is not disrupted by molecules of the air e.g. oxygen and water. For this reason, it is impossible to view living specimens. This is the principal limitation of electron microscope images. They show the structure of dead cells which have been subjected to mechanical trauma in the preparation process and are held in a vacuum.

Specimens are prepared by immersion in a series of increasingly pure solutions of alcohol which serve to dehydrate the cell components, then they are typically embedded in a block of resin which is cut into very thin sections by a machine resembling a high tech. bacon slicer called a microtome with a diamond or glass cutting surface. As most organelles and subcellular components are built up from low atomic number elements, the difference in density is not pronounced between one structure and another. To improve the contrast of these pictures, it is common to 'stain' the specimen with the salts of heavy metals such as lead and uranium which increase the scattering of the electrons where the 'stains' are taken up.

In the **scanning electron microscope (SEM)**, the beam of electrons passes back and forth across the surface of the prepared specimen producing a three dimensional effect. This technique is used to show surface features.

Differential centrifugation

The function of individual organelles has been established largely by isolating each organelle from the rest of the cell components and subjecting it to conditions which copy those which are thought to exist in the natural state. Naturally, the most detailed functional information has resulted from the organelles which are most easily isolated, namely mitochondria and chloroplasts. The isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments, typically by breaking up fresh tissue in a test tube homogeniser. A pH buffer and sucrose solution are added to keep the cell fragments at optimum pH and ensure that the water potential of the surrounding liquid remains the same as organelles. Enzyme action and bacterial activity is slowed down by immersing tubes containing cell fragments in ice.

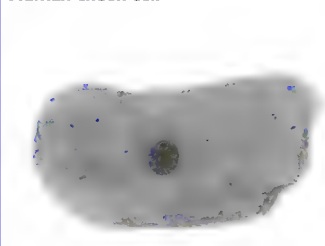
The fragments are separated by a series of spins in a high speed centrifuge (ultracentrifuge) in a process called differential

◆ CHECKPOINT SUMMARY

- ◆ Electron microscopy reveals greater detail of cell structure than the light microscope
- ◆ Resolution is the ability to see detail. The higher the resolution the more of the detail present can be seen
- ◆ Magnification is enlarging in size. Increasing magnification only reveals extra detail if the detail can be resolved
- ◆ Resolving power of light microscope limited by the wavelength of light. Put simply some detail is so small that it cannot be 'hit' by light
- ◆ Endless magnification of the image produced by a light microscope yields no extra information
- ◆ Wavelengths of electron beams much shorter than that of light and give the electron microscope much greater resolving power than the light microscope
- ◆ Therefore extra magnification up to $\times 250\,000$ yields equivalent amount of detail
- ◆ The risk of distortion and the production of artefacts is greater with the EM as a result of the total dehydration, very thin sectioning, staining, and high temperatures generated
- ◆ Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section
- ◆ The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells
- ◆ The transmission electron microscope beams electrons through very thin specimens
- ◆ Mechanical disruption of the cells produce subcellular fragments. The fragments are separated by a series of spins in a high speed centrifuge (ultracentrifuge) in a process called differential centrifugation (or 'cell fractionation'), isolating different organelles
- ◆ The heaviest components separate at the lowest spinning speeds, but centrifuged 'organelle bands' not completely 'pure'
- ◆ Cells specialised for particular functions show organelles developed in similar way, e.g. active voluntary muscle, and secretory cells, have increased number of larger mitochondria.

centrifugation (or 'cell fractionation'). The heaviest components separate at the lowest spinning speeds. At speeds 1000 times the pull of gravity, the cell nuclei separate out into a pellet at the bottom of the tube. The remaining liquid (supernatant), containing all the other cell fragments may then be transferred to another tube and recentrifuged at a higher speed. Mitochondria form a pellet at about 3000 times gravitational pull and the smaller cell fragments require even greater speeds. Isolated fragments are never pure, particularly the smaller components such as lysosomes because the cell membrane systems (ER, Golgi apparatus, nuclear and plasma membranes) reform into small vesicles of similar size when cells are broken up. Isolated cell fragments may be purified further by centrifugation in tubes which are prepared with concentrated gel-like solutes in the form of a density gradient so that the solution at the top of the tube is less dense than that at the bottom. As the tube is spun with the selected sample, so the cell fragments separate in bands at a level in the tube corresponding to their own density.

Human cheek cell



Cell differentiation - tissues and organs

When cells are first formed by the process of division they all look much alike. As different genes are 'switched' on and off groups of cells (**tissues**) become specialised for a particular function. The process of cell specialisation is called **differentiation**. The columnar epithelial cell described earlier shows particular specialisation for the uptake of materials across its outer membrane. Similar cells form a lining (epithelial) tissue in the small intestine of the gut. Tissues are made up of similar cells with a common function or functions.

Human cheek cells from inside the mouth come away easily because they form the outer layer of a multi-layered (compound) tissue which is replenished continually by the formation of new cells from the base layer. Individual cells are flattened (squamous), and these cells belong to a type of tissue called **squamous epithelium**.

Single layered squamous epithelium is found forming the walls of the air sacs (alveoli) in the lungs, and the Bowman's capsule of the kidneys.

Plant cells are also grouped into tissues. For example the elongated cells of the xylem (water conducting and supporting tissue) and phloem (sucrose transporting tissue) are arranged end to end to form specialised vascular tissue running up and down the stem.

Organs are made up of different tissues organised to carry out a particular function or related functions, for example the leaf is an organ specialised for photosynthesis. Within it the palisade mesophyll tissue is the main photosynthetic region, the spongy mesophyll tissue aids gas exchange, and the vascular tissue allows transport of water, minerals and organic materials e.g. sucrose.

In a similar way, the small intestine may be regarded as an organ, made up of a variety of tissues, epithelial tissues, connective tissues, glandular tissues and muscle tissues.

◆ CHECKPOINT SUMMARY

- ◆ The cells of multicellular organisms may differentiate and become adapted for specific functions
- ◆ In multicellular organisms cells are organised into tissues. A tissue being an association of similar cells specialised and coordinated to a particular function. Some functions being more specialised than others
- ◆ Cells in the same tissue develop from the same original cell type, even though the fully differentiated cells may appear very different
- ◆ Examples of tissues in mammals include epithelium covering surfaces e.g. columnar epithelial cells lining the small intestine
- ◆ An example of a tissue in flowering plants is the palisade mesophyll found under the upper surface of the leaf
- ◆ The leaf palisade mesophyll cell is specialised for the purpose of photosynthesis. It contains numerous chloroplasts which, if living cells are observed, can be seen moving within the cytoplasm to positions favouring optimum light absorption
- ◆ Columnar epithelial cells are specialised for the purpose of transport and the membrane across which absorption occurs is adapted to provide a huge surface area by the possession of extensions called microvilli
- ◆ Different tissues become 'organised' into organs specialised to particular functions, e.g. a lung is an organ and consists of squamous epithelium, ciliated epithelium, connective tissue, blood vessels etc.

1.3

Cell Transport

Content:

Plasma membranes

- ▼ The fluid mosaic model of cell membrane structure.
- ▼ The function of proteins in membranes as receptors and carriers

Diffusion

- ▼ The effect of surface area and distance on the rate of diffusion.
- ▼ The role of carrier and channel proteins in facilitated diffusion

Osmosis

- ▼ Osmosis as a special case of diffusion across a partially permeable membrane, net movement of water depending on difference in water potentials.
- ▼ Hypotonic, hypertonic and isotonic solutions, and the importance of ion concentration in maintaining cell turgor.

Active transport

- ▼ The movement of molecules or ions through a membrane by carrier proteins against a concentration gradient.

PLASMA MEMBRANES

The cell surface membrane (**plasmalemma** or **plasma membrane**) and the membranes which enclose the cell organelles and make up the extensive system of interrelated channels and vesicles within the cell share the same structure. It is a structure which is so flexible that it can break and reform easily yet it is capable of performing complex functions, acting as a barrier, container, transport regulator and as a site of recognition, distinguishing between 'friendly' and 'foreign' substances, 'self' and 'non-self'.

Membranes are constructed from lipid, protein and carbohydrate components. The structure is based on a bilayer of phospholipid molecules, strengthened by cholesterol. Phospholipids adopt this bilayer structure automatically in fluid surroundings because the fatty acid 'tails' of the molecules are water repelling (hydrophobic) whilst the phosphate/alcohol 'heads' are water attracting (hydrophilic).

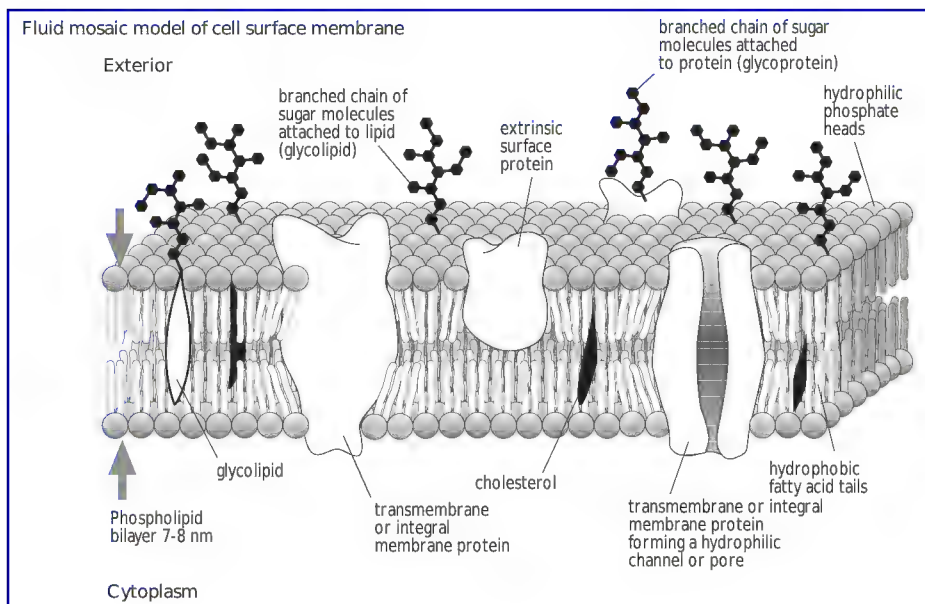
Proteins of various sizes and shapes are located on and in the bilayer. Some are bonded to the hydrophilic head region and do not penetrate the interior of the membrane (**extrinsic proteins**), others, have one end buried within the fatty layer and one end sticking outside of the cell membrane (**intrinsic proteins**). A third kind, (**transmembrane proteins**), pass all the way through the membrane to create special channels. The carbohydrate component of cell

membranes consists of short polysaccharide chains joined to membrane proteins or fats forming **glycoproteins** and **glycolipids** respectively, which project outside the cell membrane.

Individual molecules of phospholipid and protein in the membrane have a degree of **mobility** within the membrane. This fluidity, coupled with the bubbled appearance of the proteins on the surface of membranes gave rise to the the term '**fluid mosaic model**' when the structure was first proposed. Cholesterol stabilises membranes by limiting the movement of phospholipid molecules within the membrane.

Cells have the ability to sense and respond to the presence of a great variety of molecules. This ability is due to proteins in the surface membrane which act as **receptors**. Hormones such as insulin and adrenaline, and many drugs and toxins lock onto recognition sites on receptor proteins triggering changes in the cell's behaviour.

Glycoproteins and glycolipids have a very important role in personalising a cell's identity. They are orientated in the membrane bilayer with the chain of sugar molecules to the outside. The possibility for variation in the structure and different combinations of these chains gives an endless range of different surface patterns, and forms the basis of a recognition system. Each individual has his or her own cell surface 'print'. 'Self' recognises 'self', and immune systems are mobilised when 'non-self' substances are encountered. The glycoproteins and glycolipids of the cell membrane are responsible for an individual's tissue type, a well known example of which is the blood groups.



The plasma membrane acts as a partially permeable barrier to the passage of substances into and out of the cell, for example water and fat soluble substances pass through more easily than sucrose. The different rate of passage of electrically charged ions generates membrane potentials which in turn influence the further uptake of charged ions, and are also involved in cell sensitivity, particularly in nerve cells.

The transport of respiratory gases, nutrients and metabolic waste products occurs through the membrane, either directly through the bilayer, or via channels or 'pores' created by transmembrane proteins. In some cases the substances move down a concentration gradient (i.e. from high concentration to low concentration) which is known as **passive transport**. In others, substances are moved against the concentration gradient (i.e. from low concentration to high concentration) in a process requiring energy in the form of ATP, which is known as **active transport**.

DIFFUSION and the FACTORS which DETERMINE its RATE

Passive diffusion

Molecules and ions may move passively by diffusion, which is defined as the overall (net) movement of substances from regions of their higher concentration (higher chemical potential) to regions of their lower concentration (lower chemical potential) until equilibrium is reached. The movement of the particles of substances in diffusion is random in all directions, as a result of their inherent kinetic energy. If there are more in one area than another, the net effect is for the particles to distribute themselves equally throughout that particular space, i.e. the concentration gradient evens out until at equilibrium there are an equal number of particles moving in all directions.

The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the **concentration gradient**), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed in the following equation known as **Fick's law**.

$$\text{Rate of diffusion} = \frac{\text{difference in concentration} \times \text{surface area}}{\text{thickness of the exchange surface}}$$

In principle, the greater the surface area, the greater the concentration difference, and the thinner the separating layers, the greater the rate of diffusion.

The most important factor affecting diffusion across cell membranes, however, is the chemical nature of the substance being transported. Diffusion across the bilayer depends principally upon the solubility of the substance in fat. You will remember that the phospholipid bilayer is strongly hydrophobic. Polar molecules such as glucose, some amino acids, and inorganic ions pass through very slowly or not at all, whilst non polar molecules such as calciferol (vitamin D) move across with ease. It is interesting to note that the first anaesthetics (chloroform and ether) were fat solvents that disrupted membrane structure and function especially in nerve cells.

◆ CHECKPOINT SUMMARY

- ◆ All cell membranes are thought to share the same structure (except the inner membrane of mitochondria).
- ◆ This is based on a lipid bilayer formed from phospholipids aligning themselves with their hydrophobic poles next to each other within the membrane, and hydrophilic poles facing outwards
- ◆ This fluid arrangement is stabilised by the presence of another lipid - cholesterol.
- ◆ Various proteins are embedded within this lipid bilayer
- ◆ Extrinsic proteins are found on the surface of the membrane
- ◆ Intrinsic proteins have one end embedded in the lipid bilayer and the other protruding out of the external surface of the membrane
- ◆ Transmembrane proteins span the lipid bilayer and create aqueous channels of pores through which water soluble substances can pass by diffusion
- ◆ Short polysaccharide chains attached to proteins (glycoproteins) and to fats (glycolipids) protrude from the external surface and act as cell recognition sites (receptors or antigens)
- ◆ The patchwork arrangement of all these types of molecules in the lipid bilayer is the origin of the description 'mosaic'.
- ◆ The cell surface membrane defines the limits of the cell
- ◆ It receives 'messages' by providing receptor sites for hormones, cell recognition of 'self' and 'non-self', and act as antigens with which antibodies can bind
- ◆ It acts as a partially permeable membrane regulating the transport of substances across the membrane
- ◆ Internal membrane systems provide a surface for attachment of organelles and enzymes, and compartmentalise specialised functions within the cell.

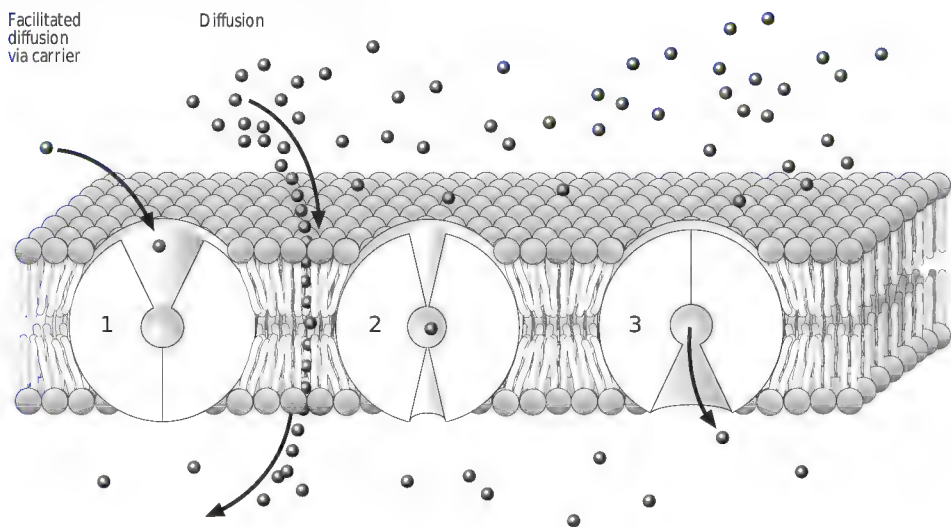
Facilitated diffusion

The transport of polar molecules e.g. glucose, some amino acids, and inorganic ions, across cell membranes, occurs through hydrophilic channels formed by transmembrane proteins, in a process called **facilitated diffusion**. Polar molecules also become temporarily attached to special binding sites on transmembrane proteins called **carriers**. These are rather like enzymes in that they are shape specific and accelerate a process which is already 'downhill' in terms of energy. All that is needed is a concentration gradient. The attachment of a molecule to the binding site alters the overall shape of the carrier in such a way as to deposit the molecule on the other side of the membrane along their normal concentration gradients.

As has been seen, the transport of inorganic ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and chloride (Cl^-) across cell membranes depends upon transmembrane proteins.

Some transmembrane proteins form selective **ion channels** through which the facilitated diffusion of ions occurs down their concentration gradients. These ion channels cannot be coupled to an energy source, and cannot be involved in active transport across the membrane.

Diagram to show diffusion and facilitated diffusion



Osmosis

Cell membranes are **partially permeable**, allowing the passage of substances at different rates. Generally cell membranes are more permeable to water than to many solutes.

Water moves according to the same principles of diffusion, but it is not usual to refer to high and low 'concentrations' of water - the term 'concentration' being traditionally associated with any solutes dissolved **in** water in a solution. Therefore the preferred term is the chemical potential of water or **water potential**.

Water always moves (diffuses) from a region of higher water potential to one of lower water potential. You could think of water potential as the **tendency for water molecules to move** i.e. diffuse. The higher the water potential the more easily will the water molecules move, and the lower the water potential the less easily will the water molecules move.

At standard pressure and temperature, pure water has the highest water potential. If substances are dissolved in solution then the water potential is decreased because the solutes associate with water to a greater or lesser extent and reduce the ease with which the water molecules can move. Therefore any solution has a lower water potential than pure water, and a more concentrated solution has a lower water potential than a less concentrated solution.

Water potential of a fluid can be described as the tendency of water to move into it from pure water. If the fluid **is** pure water, there is no movement, so the **water potential of pure water is zero**. The water potential of any solution is less than pure water and is therefore negative. The more negative the water potential the greater the tendency of water to move into that system.

Where a solution is separated by a partially permeable membrane from pure water or a less concentrated solution, water will diffuse into the more concentrated solution until equilibrium is reached. The special case of the diffusion of water across a partially permeable membrane is known as **osmosis**.

Movement of water between plant cells and between them and their environment in terms of water potential

In plant cells a non-living cellulose cell wall surrounds the cell surface membrane. The cell wall is freely permeable to water and dissolved substances (solutes.) By contrast, the cell surface membrane is partially permeable, restricting the movement of solutes but allowing water to diffuse in and out more freely. Where a solution is separated by a partially permeable membrane from pure water or a solution of different concentration, the movement of solutes by diffusion is restricted and water will diffuse from the less concentrated (**hypotonic**) into the more concentrated (**hypertonic**) solution until equilibrium is reached.

Water will always tend to move from regions of high water potential to regions of low water potential. Where a plant cell is surrounded by a solution with a higher water potential than that of the cell contents (protoplast), water will move into the cell. As it does so, the cell contents expand until the tendency for water to enter is counterbalanced by the force exerted by the stretched cell wall. At

this equilibrium point the cells are described as fully **turgid**. In a well watered plant, the turgid cells exert pressure on each other making the whole structure rigid (especially the spongy mesophyll in leaves.) Loss of water causes a loss in turgor, the cells become **flaccid**, and the leaves wilt.

In the very unusual case of the surrounding water having a lower water potential than the plant cells (for example, where the soil is flooded with salty water) water will move out of the cell by osmosis so that, in extreme cases the membrane shrinks away from the cell wall. Cells in this state are said to be plasmolysed. (Note that all plasmolysed cells are flaccid, but not all flaccid cells are plasmolysed.)

All these considerations affect the movement of water between plant cells and between them and their environment. For example root hair cells gain or lose water in relation to the relative water potentials of their contents and the soil solution, and leaves lose water to the atmosphere according to the same principles.

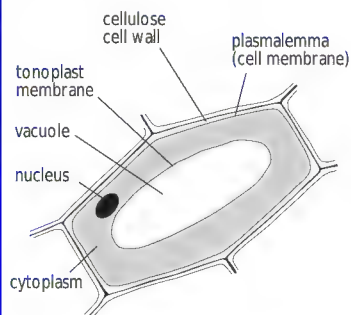
Active transport

Some transmembrane proteins act as active **ion pumps**, which use energy from respiration, to move ions against their concentration gradients. The ion channels can open and close like 'gates', and the activity of the ion 'pumps' can be varied. In these ways, the membrane can be made selectively permeable to certain ions at certain times.

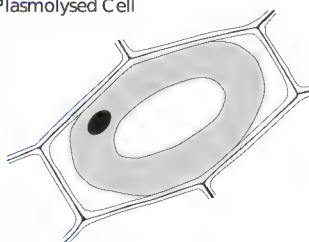
As a result of the distribution of ions on either side of the membrane **transmembrane potentials** are created, in which there is an overall difference in electric charge between the inside and the outside of the membrane. In normal conditions, the inside of the cell membrane is negatively charged relative to the outside, and this will assist the uptake of positively charged ions (cations) and oppose the uptake of negatively charged ions (anions). Thus the movement of ions across membranes is influenced by both electrical and chemical gradients i.e. **electro-chemical gradients** (these have particular importance for the function of nerve and muscle cells).

Some of these transmembrane protein carriers carry two substances at once in a **coupled mechanism**. A common example is the sodium/potassium pump (Na^+/K^+ pump), in which the active pumping of sodium out of a cell is coupled with the pumping-in of potassium, both against their diffusion gradients (for every two sodium ions pumped out, three potassium ions are pumped in, resulting in high potassium and low sodium levels within the cell). Another example is where the sodium pump is linked to the uptake of glucose. The sodium ions are pumped actively out of the cell and diffuse back into the cell via a carrier associated with glucose. In this case the glucose is moving passively down its concentration gradient, but at a faster rate as a result of its association with the re-entry of sodium which is kept at a high rate by its being continually pumped out of the cell.

Normal Cell



Plasmolysed Cell



◆ CHECKPOINT SUMMARY

- ◆ Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores
- ◆ Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move)
- ◆ At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative).
- ◆ Facilitated diffusion occurs via specialised carriers in the membranes which reduce the resistance to the diffusion of these substances
- ◆ Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient
- ◆ Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.

1.4

Organisms Exchange Materials with their Environment

Content:

Surface area: volume ratio

- ▼ The relationship between the size of an organism or structure and the surface area: volume ratio, and the significance of this for the exchange of substances and of heat
- ▼ Changes of body shape and the development of systems in larger organisms to facilitate exchanges as the ratio decreases.

Gaseous exchange

- ▼ The development of internal gas exchange surfaces in larger organisms to maintain adequate rates of exchange.
- ▼ The structure, location and adaptation for function of the gas exchange surfaces and related structures in:
 - ▼ dicotyledonous plant leaves (mesophyll and stomata)
 - ▼ bony fish (gill lamellae and filaments, including the counter current principle)
 - ▼ mammals (alveoli, bronchioles, bronchi, trachea, lungs)

Ventilation

- ▼ The ventilation systems in bony fish and mammals

SURFACE AREA TO VOLUME RATIO

As an organism or structure increases in size the ratio of its surface area to its volume decreases. For a given size the shape with the smallest surface area to volume ratio is a sphere, and this ratio can be increased by flattening and sub-dividing the structure as much as possible.

The surface area to volume ratio of organisms and structures is significant for the exchange of substances and of heat with the surroundings.

All living cells obtain oxygen and nutrients from, excrete waste substances into, and gain or lose heat from the fluid which surrounds them. In the case of single celled organisms, the fluid is the environment in which they live, normally fresh or salt water. The individual cells of multicellular organisms are also surrounded by a fluid medium called **tissue fluid** or extracellular fluid, with which they exchange materials. This is referred to as the 'internal environment' and exchanges then occur between the internal and the external environment in larger organisms.

Unicellular and small multicellular organisms have a large surface area relative to their volume, and all of their cell contents are close enough to the surrounding medium for sufficient exchanges by diffusion to meet their needs. They do not require specialised exchange surfaces linked to an internal transport system. In

unicellular and small multicellular organisms oxygen, carbon dioxide and waste in the form of ammonia may be exchanged and transported by simple diffusion.

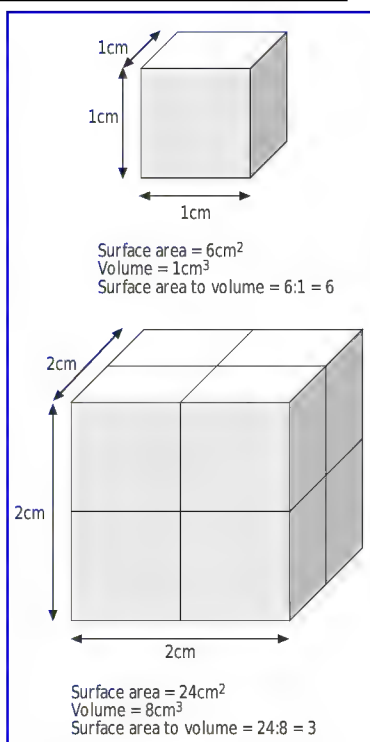
As the surface area to volume ratio reduces with increasing size, exchanges and internal transport by diffusion are facilitated by changes in body shape and the development of systems. For example in the Cnidaria (e.g. jellyfish, sea anemones, *Hydra*) there is a large body cavity which is filled with the external medium (water); in the Platyhelminthes (the 'flat' worms e.g. the tape worms and liver flukes) there is a flattened body shape; and flowering plants have leaves with a flattened form with large internal air spaces.

In large multicellular organisms there is simply not enough external surface area, relative to their volume, to provide for adequate exchange with the environment. Similarly the volume is too large to be supplied by simple diffusion from the surface.

In these organisms, gas exchange occurs in specialised exchange organs with large delicate surface areas, which are protected from damage, often by being internal e.g. gills of fish, and lungs. Internal mass transport systems carry out the rapid and efficient transport of substances to and from the external medium, and between the different tissues and organs.

Internal transport systems which move blood past the exchange surface, and ventilating mechanisms which move the external medium over them, maintain the diffusion gradients across the exchange surface.

With regard to heat exchange, most organisms are unable to regulate their body temperature and are in equilibrium with their surroundings, birds and mammals do regulate their body temperature independently of the surroundings. With decreasing size the energy requirements and the metabolic rate increases, as an increasing proportion of the heat generated is lost over the surface area, so that there is a minimum size for birds and mammals.



Gaseous exchange

Gas exchange is the term used to describe the process by which organisms exchange oxygen and carbon dioxide with the environment (although in the aquatic environment the 'gases' are in solution).

Dicotyledonous plant leaves (mesophyll and stomata)

As mentioned above, the flattened shape of plant leaves increases the surface area of a given volume. In addition leaves have a large internal surface area specialised for gas exchange as a result of the large intercellular spaces in the **mesophyll** tissue. (Dicotyledonous plants are those which have broad leaves with a branching pattern of veins and develop from seeds with two embryonic 'leaves' called cotyledons. They are distinguished from monocotyledonous plants which have grass type leaves with parallel leaf veins and only one seed cotyledon.)

The **palisade mesophyll** tissue is made up of columnar cells under the upper epidermis with large vertical intercellular spaces, and is the main photosynthetic layer of the leaf.

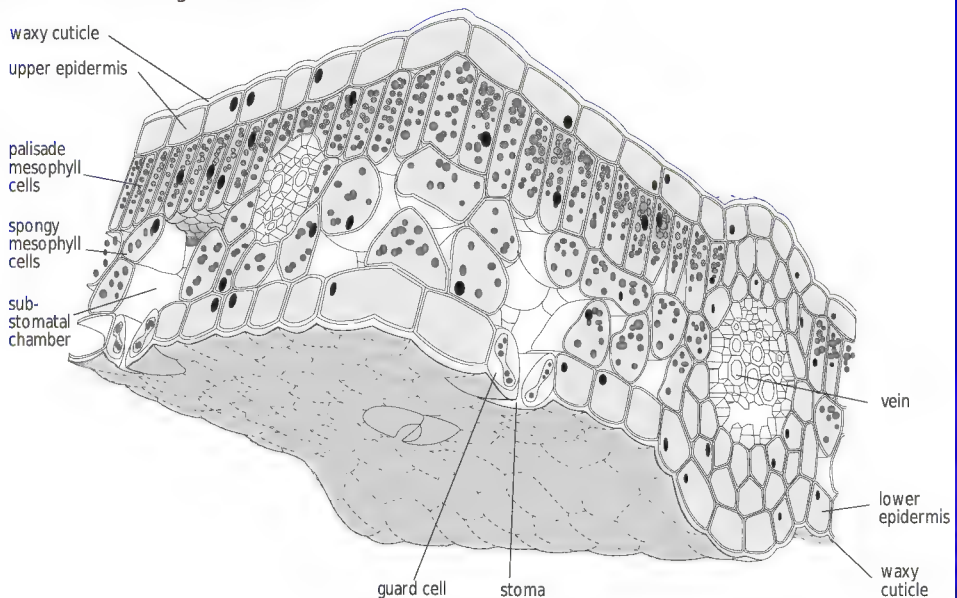
The **spongy mesophyll** tissue is made up of relatively large irregular cells. They are loosely packed, and there is an interconnecting network of intercellular air spaces.

The surface of the leaf is covered by a waterproof cuticle through which some gaseous exchange can occur, but virtually all gaseous exchange is via holes in the epidermis known as stomata (singular = stoma).

◆ CHECKPOINT SUMMARY

- ◆ Surface area increases with size but surface area to volume ratio decreases
- ◆ SA/V increased by flattened body shapes and structures
- ◆ Where SA/V sufficiently large, simple diffusion suffices for exchanges with environment and internal transport.
- ◆ Discussions of development of transport systems also involve level of complexity of organism
- ◆ Development of transport systems involves pumping hearts and tubes
- ◆ Development of transport systems involves development of specialised exchange surfaces with large surface areas
- ◆ Diffusion still important in all exchanges, but not in transport throughout volume of organism.

Section of leaf showing internal structure



Two specialised **guard cells** regulate the opening and closing of each stoma. The guard cells are banana shaped with specially thickened facing walls. The mechanism by which guard cells open and close stomata depend on the changes in the shape of the guard cells due to changes in their water content or turgidity. When the guard cells are distended with water, or turgid, they bend outwards due to the unequal thickening of their walls and the stomata are opened; when the guard cells lose turgor (become flaccid) the stomata reduce in size and are eventually closed.

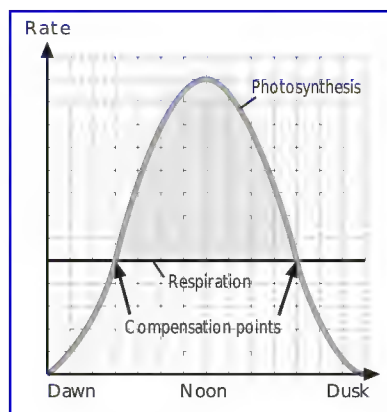
The presence of chloroplasts in most guard cells could indicate that photosynthesis is involved in some way. The simple explanation is that the guard cells produce sugars in the light by photosynthesis, and that these sugars increase the concentration of the cell contents so that water is drawn in by osmosis. This increases their turgidity and results in the opening of the stomata. However, this cannot explain the opening and closing of all stomata since many stomata open at night, and changes in the rate of sugar production by photosynthesis would be too slow to account for the rapid opening sometimes seen. It is thought that the mechanism is, at least to some degree, an active process which involves potassium ion transport across the cell membranes of the guard cells.

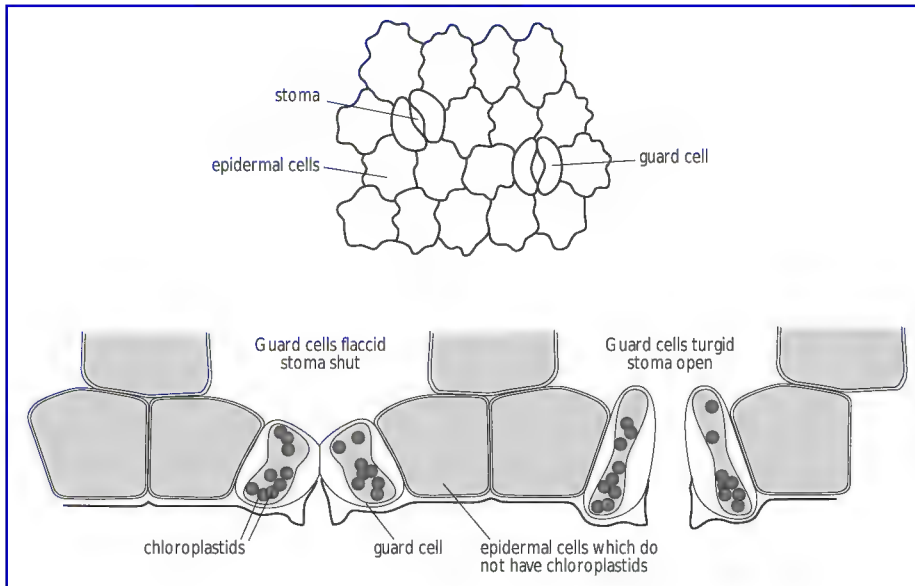
Sub-stomatal cavities link to the extensive intercellular air spaces of the mesophyll tissues.

The air in the air spaces is refreshed by exchanges when the stomata are open. There are no 'breathing movements' so the rate of exchange is determined entirely by diffusion gradients, other than when the leaves are buffeted by high winds. Carbon dioxide and oxygen in the air dissolve in the thin layer of water coating the surface of the spongy cells, then diffuse through the cell wall and the cell membrane.

In plants the predominate direction of movement of oxygen and carbon dioxide depends upon the relative rates of respiration and photosynthesis. Oxygen is absorbed when the rate of respiration exceeds the rate of photosynthesis (in the dark), and released when the rate of photosynthesis exceeds the rate of respiration (in the light). Carbon dioxide is absorbed when the rate of photosynthesis exceeds the rate of respiration (in the light), and released when the rate of respiration exceeds the rate of photosynthesis (in the dark).

When the rates of respiration and photosynthesis are the same, (compensation point) there are no net (overall) exchanges of oxygen and carbon dioxide.





Bony fish

The amount of oxygen available to living organisms depends on its relative proportion in the surrounding medium. Air is a much more favourable environment than water in this respect because it contains 21% oxygen whilst the amount dissolved in water is much smaller. Furthermore, the amount of oxygen dissolved in water varies greatly according to the temperature and pressure, the warmer the water, and the lower the pressure, the less oxygen it can contain.

Temperature °C	Volume of oxygen cm^3/dm^3
0	10.29
10	8.02
15	7.72
20	6.57
30	5.57

The most successful aquatic species, however, have become adapted to make best use of the available oxygen. The most advanced and efficient system for gas exchange in water is exemplified by bony fish (the term 'bony' is used to distinguish fish with a bone skeleton e.g. trout, cod from those which have a cartilaginous skeleton e.g. sharks, rays).

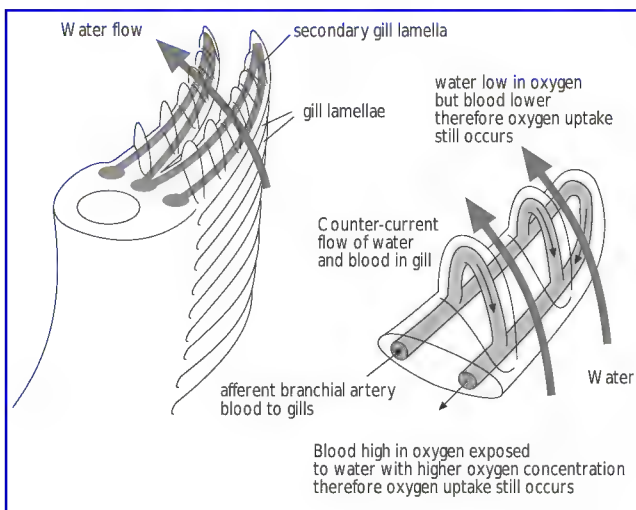
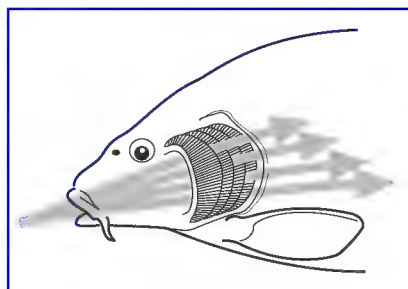
The gas exchange structures in bony fish

The organs of gas exchange in the fish are gills, four of which lie on each side of the pharynx supported on curved bones called **gill arches**. Each gill is made up of about 70 leaf like flaps called **gill lamellae**, and the surface area of the lamellae is further increased by **secondary lamellae** which project at right angles in the direction of the water flow. Small comb like extensions of the gill arches, the **gill rakers**, prevent larger particles in the water passing through the gill slits and damaging the delicate gas exchange surface. The gill lamellae provide a huge surface for gas exchange and they are well supplied with blood capillaries for the transport of oxygen and carbon dioxide.

You will notice from the diagram that the blood flows in the opposite direction to the water, a principle referred to as the **counter current principle**. This promotes an efficient exchange of gases because it ensures a uniformly high diffusion gradient all the way along the length of the secondary lamella. If the flow of blood was in the same direction as the water (parallel flow) the diffusion of gases would be rapid at first due to fresh, oxygen loaded water meeting deoxygenated blood high in carbon dioxide. However, further along the filament, the water and blood would be in equilibrium with equal levels of oxygen and carbon dioxide, so exchanges would slow down or stop altogether.

Counterflow ensures that as much oxygen as possible diffuses from the water into the blood of the fish, and as much carbon dioxide as possible is transferred in the opposite direction.

Air is much easier than water to move over a gas exchange surface, and it has a much higher oxygen content. Oxygen diffuses about three million times faster in air than in water. However, any large, thin, permeable surface, well supplied with blood exposed to the air will lose water by diffusion and evaporation. This poses a problem for all terrestrial organisms.

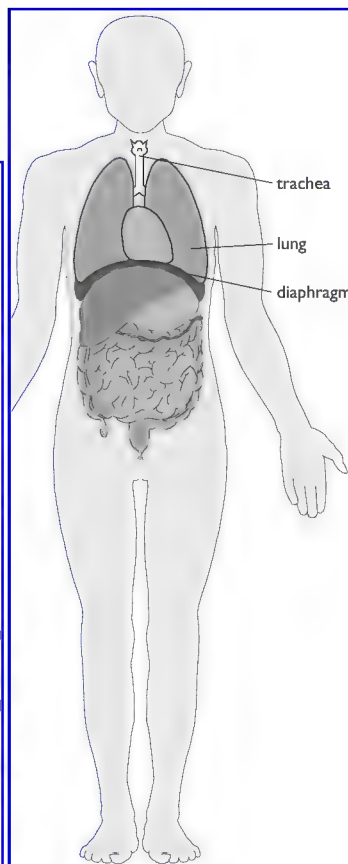
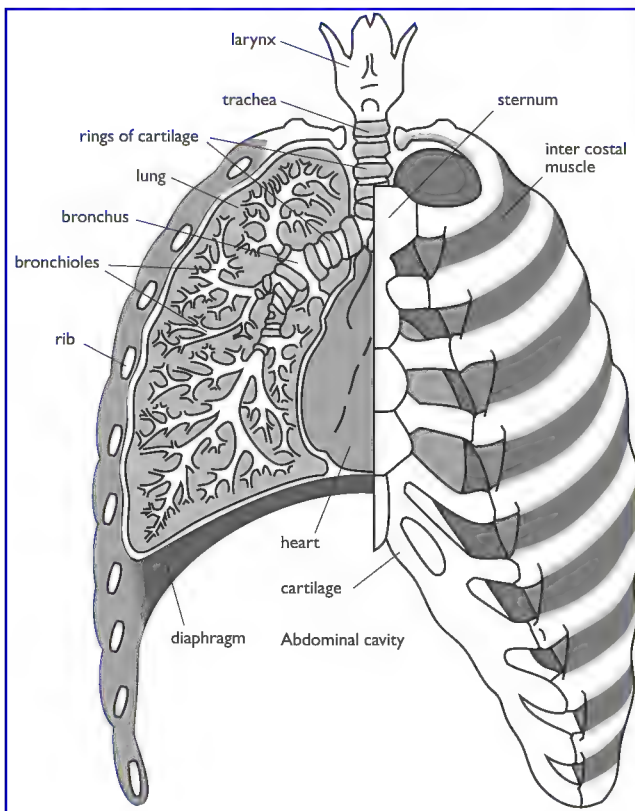


◆ CHECKPOINT SUMMARY

- ◆ Larger and more complex organisms develop internal gas exchange surfaces to maintain adequate rates of exchange
- ◆ Dicotyledonous plant leaf has an internal gas exchange surface at the surface of the mesophyll tissue, mainly the spongy and to a lesser extent the palisade
- ◆ Oxygen and carbon dioxide are exchanged here by diffusion to and from the large intercellular air spaces which link to the sub-stomatal cavity.
- ◆ The sub-stomatal cavity exchanges gases with the external air via the stomata
- ◆ Stomata typically open in the light and close in the dark. In the light carbon dioxide is absorbed for photosynthesis and oxygen and water vapour is lost
- ◆ Bony fish have gill lamellae and filaments on gill arches which provide a huge surface area for gaseous exchange
- ◆ There is one-way through-flow of water over the gills, and the blood in the vessels of the gill lamellae flows in the opposite direction. This countercurrent flow maintains the diffusion gradients of oxygen and carbon dioxide and enables gas exchange to take place over the whole surface of the gills.

The gas exchange structures in mammals

The organs of gas exchange in mammals are the lungs. Together with the heart, they fill all the available space in the air tight **thoracic (chest) cavity** bounded by the ribs and the muscular sheet known as the **diaphragm**.



A series of tubes, the **trachea, bronchi and bronchioles** form a system of branching airways referred to as the 'bronchial tree'. The wall of the trachea and bronchi are composed of four layers. These are:

- ▼ the inner mucous membrane or **epithelial** layer;
- ▼ a **submucosa** layer consisting of connective tissue, elastic fibres and mucous glands;
- ▼ a layer of **smooth muscle** and **cartilage**;
- ▼ a tough outer coat of **connective tissue**.

Bronchioles differ in that they lack cartilage and do not have mucus glands present in the submucosa. In very narrow bronchioles the smooth muscle and elastic fibres may form a single layer.

Cartilage supports the trachea and bronchi and prevents their collapse. In the trachea the cartilage forms 'C-shaped' rings which are arranged so that the open ends of the C are at the back of the tube, facing towards the spine. These support the tube and keep it open during inspiration (breathing in), and neck twisting. Between the ends of the Cs at the posterior (back) of the trachea, smooth muscle completes the tube. The incomplete nature of these 'rings' of cartilage allows for compression when the oesophagus expands as food is swallowed. In the bronchi the cartilage does not form rings but instead forms plates within the smooth muscle layer to strengthen the tubes.

Involuntary (smooth) muscle found in the trachea, bronchi and bronchioles also helps maintain the shape of the tubes and prevents collapse of the airways. The smooth muscle receives nerve impulses from the autonomic nervous system which can regulate the diameter of the tubes, and therefore the flow of air through the system. This is significant in exercise where increased air flow is required. In diseases like asthma and anaphylaxis excessive constriction of the bronchioles occurs restricting air flow, sometimes with fatal results.

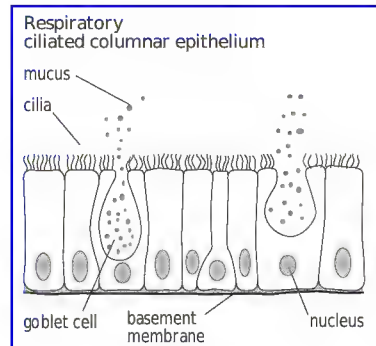
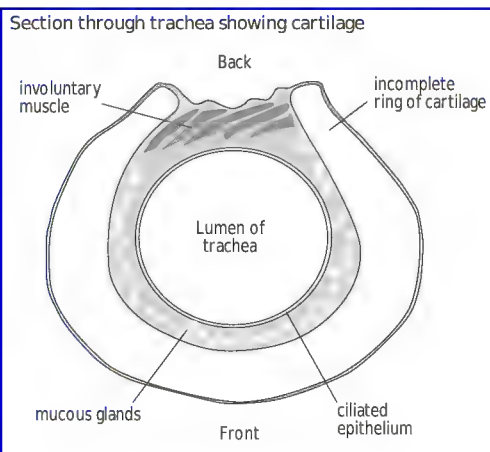
Epithelium lining the trachea, bronchi and bronchioles is known as the mucous membrane, and consists of ciliated columnar epithelium. This possesses two main cell types: ciliated cells and goblet cells. At the end of the bronchioles, close to the alveoli no goblet cells are found, only ciliated cells.

Goblet cells which are interspersed amongst the ciliated cells in the trachea and bronchi, are responsible for producing and secreting mucus within the respiratory tract. The layer of mucus which lines the airways traps dust and bacteria. It may also play a role in humidifying inhaled air.

Ciliated epithelial cells have a protective function. The co-ordinated beating of the cilia sweeps the mucus coating the air passages, which contains small inhaled particles such as dust, towards the pharynx (throat) where they can be swallowed, or ejected by the cough reflex. This prevents such particles from entering the alveoli and interfering with gaseous exchange.

◆ CHECKPOINT SUMMARY

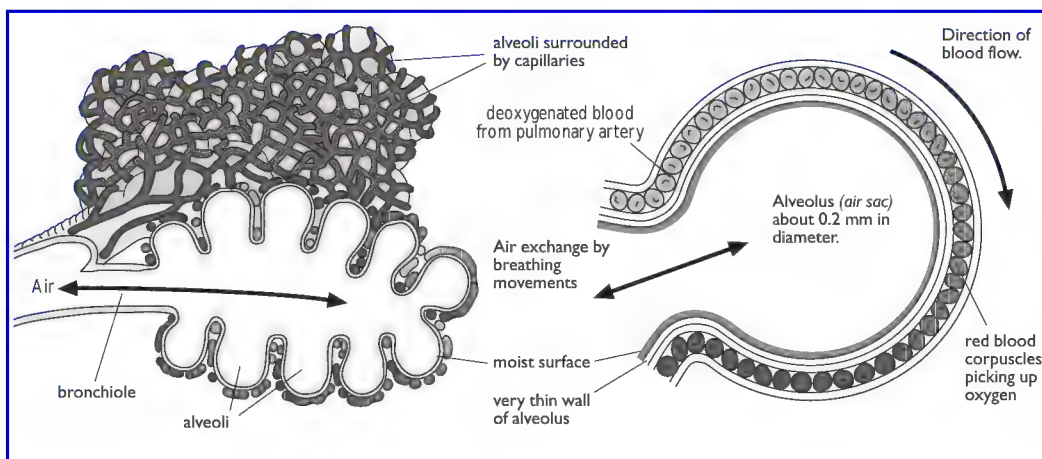
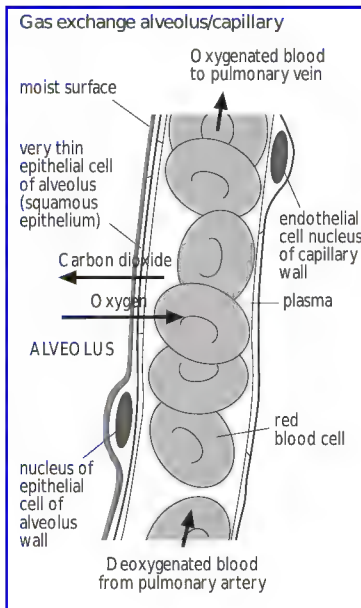
- ◆ Mammalian lungs are made up of millions of small air sacs (alveoli) which are supplied by a system of branching and progressively smaller air tubes; the trachea, bronchi, and bronchioles
- ◆ Gas exchange occurs over large surface area of alveoli which is a product of their relatively small size and large number
- ◆ Diffusion gradients of oxygen and carbon dioxide dependent upon partial pressures of gases on either side of diffusion surface.
- ◆ Diffusion surface composed of thin squamous epithelium ridged around the sandwiched capillaries
- ◆ Surface film of water an inevitable consequence of a permeable surface exposed to air decreases diffusion of oxygen more than the diffusion of carbon dioxide
- ◆ Air in alveoli remains fairly constant in composition independent of breathing cycle
- ◆ Diffusion gradients maintained by circulating blood and ventilation of lungs
- ◆ As demand for oxygen increases so does steepness of diffusion gradients and speed of circulation thus meeting extra demand.



Alveoli or air sacs are the respiratory surface where gas exchange occurs, and they make up the mass of the lungs. The ends of the bronchioles terminate in tubes, only 1-2 mm in diameter, known as alveolar ducts which lead to the alveoli. Although each alveolus is very small, the large numbers of alveoli (several hundreds of millions) provide an enormous surface area for gas exchange (70-90m² in an adult human lung).

Each alveolus is surrounded by a net of capillaries which together form the **alveolar-capillary complex**. As this name suggests, the endothelium of the capillaries is attached to the thin squamous epithelium of the alveolus via a shared basal membrane. The air inside the alveolus and the blood in the capillaries is thus separated by a distance of just the thickness of two layers of cells. This is one feature that helps rapid diffusion of oxygen from alveolus to blood and carbon dioxide from blood to alveolus. Other features include the large surface area already mentioned, and the steep diffusion gradients which are maintained between the blood and the alveolus due to the constant movement of the blood and the constant ventilation of the lungs.

A phospholipid material called a **pulmonary surfactant** acts like a detergent, reducing the surface tension of fluids at the alveolar surface, and ensuring that the tiny air sacs do not collapse during the breathing movements.



Ventilation

Animals with internal gas exchange surfaces need mechanisms for conveying gases between the environment and these surfaces. Such mechanisms are called ventilation mechanisms.

In fish the ventilation mechanisms involve muscular contractions which serve to maintain a constant flow of water over the gas exchange surfaces, but in mammals the flow of air is tidal. The process of water or air intake is called **inspiration**, and the expulsion of air or water is called **expiration**.

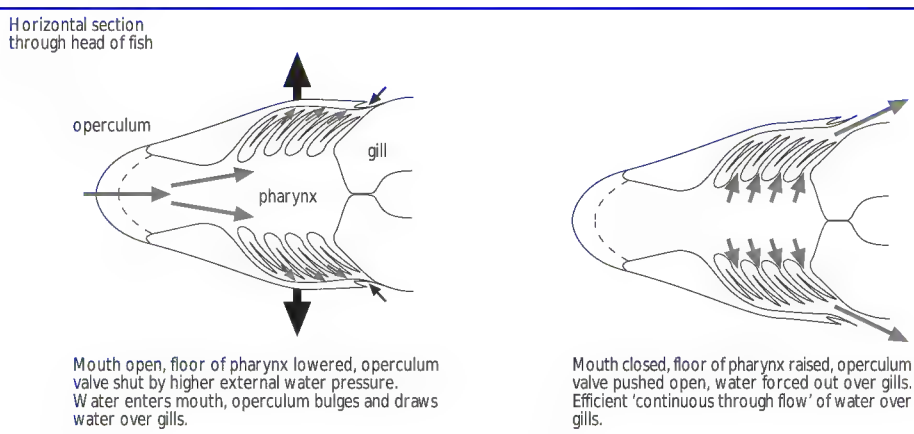
Ventilation in bony fish

Inspiration of water in a bony fish is achieved as the mouth opens and the floor of the pharynx is lowered by muscular contraction. The volume of the pharynx increases, the pressure decreases below that of the external water, the operculum valve is forced shut as the external water pressure exceeds that of the mouth and pharynx, and water enters the mouth.

When shut, the operculum bows outwards by muscular contraction, increasing the volume of the opercular cavity and decreasing the pressure, so that water continues to flow over the gills even when the floor of the pharynx is lowering and water is entering the mouth.

Expiration occurs when the mouth is shut and the floor of the pharynx is raised forcing water over the gill lamellae and out through the gill slits, pushing the operculum open.

The continuous one-way through-flow maintained by these alternating 'pumps' is a very efficient ventilatory mechanism, ensuring maximum gas exchange.



Ventilation in mammals

Ventilation in mammals is achieved by contraction and relaxation of the **intercostal muscles** of the ribs, and the muscular **diaphragm**, which are controlled by the autonomic and voluntary nervous systems.

During **inspiration** (breathing in) the external intercostal muscles contract pulling the ribs upwards and outwards. At the same time the diaphragm contracts, moving downwards. Both movements increase the volume of the thorax, reducing the pressure in the thorax below atmospheric pressure and causing air to enter, inflating the lungs.

At rest, **expiration** (breathing out) does not involve active muscle contractions, but depends upon the elasticity of the tissues of the lungs and thorax. The intercostal muscles relax, allowing the rib cage to move downwards and inwards, and the diaphragm relaxes and regains its domed shape. The volume of the thorax is reduced, the pressure increased above atmospheric, and air forced out of the lungs.

During forced breathing, for example as a result of exercise or in speech, expiration is active, with the external intercostal muscles and abdominal muscles contracting to increase the pressure within the thorax.

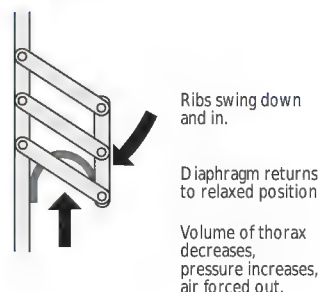
Friction between the lungs and the inner surface of the wall of the thorax is reduced by the **pleural fluid** which lies between the two pleural membranes that surround the lungs. This fluid is also under a lower pressure than atmospheric, and prevents the lungs collapsing during expiration. (If air enters the pleural cavity, either by the wall of the thorax being punctured, or alveoli bursting into it, the lung(s) do collapse, and cannot be reinflated without surgery).

Breathing movements thus result in certain volumes of air passing into and out of the lungs.

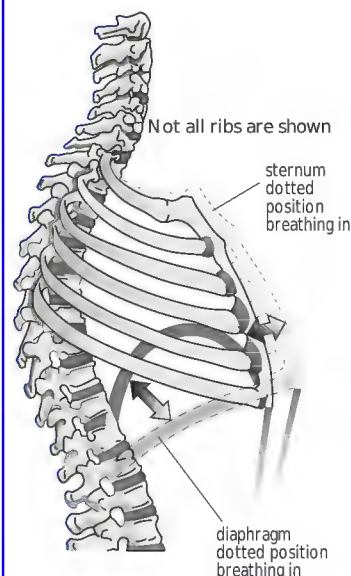
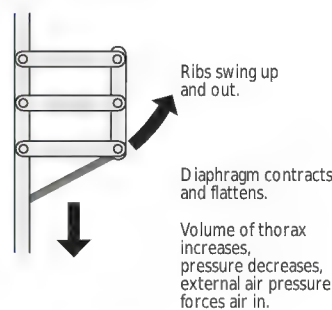
◆ CHECKPOINT SUMMARY

- ◆ Ventilation mechanisms move the external medium, e.g. air or water over the internal gas exchange surfaces, by altering the internal pressure
- ◆ In fish and mammals the ventilation mechanisms involve muscular contractions which serve to maintain a constant flow of water or air over the gas exchange surfaces
- ◆ Process of air/water intake is called inspiration, expulsion of air/water is called expiration
- ◆ Bony fish have a double pump system involving floor of the pharynx and opercula flaps
- ◆ Water enters the mouth when the internal pressure is reduced below the external water pressure, and pumped out when it is greater
- ◆ These pressure changes are achieved by alternating pumping movements of the floor of the buccal cavity and the opercula. These pumps alternate to produce a continuous one way flow of water over the gills
- ◆ With no danger of dehydration by evaporation over the thin delicate gas exchange surfaces of the gills, they can be fully exposed to the external medium
- ◆ With the lungs of terrestrial animals there is a need to maintain a humid atmosphere over the thin delicate gas exchange surfaces so they are enclosed interiorly
- ◆ Ventilation produces tidal flow in and out of a single aperture. This is less efficient than the one way flow in fish gills, as the air is not completely renewed in every cycle
- ◆ Inspiration (breathing in) - the external intercostal muscles contract, pulling the ribs upwards and outwards, the diaphragm contracts, moving downwards, the volume of the thorax increases, the pressure in the thorax decreases below atmospheric pressure causing air to enter, inflating the lungs
- ◆ Expiration (breathing out) depends upon the elasticity of the tissues of the lungs and thorax. The respiratory muscles relax, the thorax and lungs recoil as a result of their elasticity and the events of inspiration are reversed
- ◆ Elasticity of the the tissues of the lungs and thorax leads to expiration as they recoil.

Muscles Relaxed



Muscles Contracted



1.5

Enzymes

Content:

Action of enzymes

- ▼ Enzymes as proteins which act as catalysts
- ▼ The importance of enzymes in lowering activation energy
- ▼ The mode of action of enzymes - the formation of enzyme substrate complexes

Enzyme properties

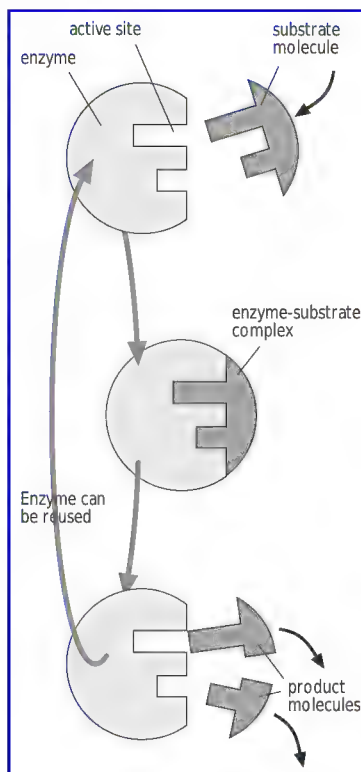
- ▼ the properties of enzymes related to their tertiary structure
- ▼ The effects of change in temperature, pH, substrate concentration and competitive and non-competitive inhibition on the rate of enzyme action.

ACTION OF ENZYMES

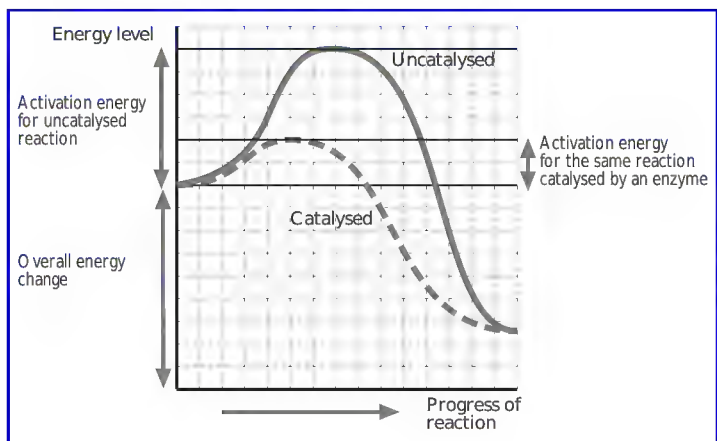
Most students of science can name an enzyme. Usually it is a digestive enzyme like amylase or pepsin, but enzymes are not just concerned with breakdown reactions like digestion, they can also help build things up. In fact, every chemical reaction in every living cell is made possible by an enzyme. Several thousand enzymes have been isolated and studied.

Enzymes are protein molecules; they are relatively large (macromolecules) of the globular type. Enzymes are **biological catalysts**. A catalyst is a substance which speeds up a reaction without being changed itself in the process. As they are not used up or changed, enzymes can be used repeatedly; therefore they are effective in relatively small amounts.

Usually enzyme molecules are much larger than the reacting molecules (substrates). In an enzyme-catalysed reaction, the substrate(s) become attached to a particular region on the enzyme called the **active site** which has a specific shape and structure matching that of the substrate(s), forming the **enzyme - substrate complex**. This association is temporary and lasts only long enough to allow the substrate molecules to interact to form the products. Once the products are formed, they are released from the active site and the enzyme is free to repeat the process.



The formation of the enzyme-substrate complex lowers the **activation energy** which is the energy needed to start a reaction. In the laboratory, it is common for reacting substances (substrates) to be heated in order to make the reaction occur more quickly. The heat energy causes the molecules to move about more rapidly (i.e. it gives them more kinetic energy) so that they collide more frequently, forming the product. In other words heat is used to overcome the activation energy. Living cells operate at a constant relatively low temperature so therefore they rely on enzyme catalysts to overcome the activation energy.



The active site matches the substrate, so different shaped substrate molecules require different shaped active sites. Most biological reactions therefore involve different, **specific** enzymes. This is possible because proteins which make up enzymes can be made in an infinite variety of shapes as a result of the nature and sequence of the amino acids and their R group.

This model of enzyme action is referred to as the lock and key model, but it is known that the shape of the active site may change as the substrate makes contact with it, adjusting to make a close fit, rather like a glove around a hand, a mode of action called **induced fit**.

ENZYME PROPERTIES

Enzymes are protein molecules with a complex tertiary structure. The way in which their peptide chains are folded and twisted into specific shapes gives each enzyme its particular qualities and mode of action. Any alteration in tertiary structure will affect the ability of an enzyme to form complexes with its substrate(s). A number of factors may affect the tertiary structure of enzymes and hence the rate of their reactions. These and other factors are considered below.

pH

This is a measure of H^+ ion concentration, which is a measure of acidity and alkalinity. A pH of 7 is neutral, less than 7 is acid, and more than 7 is alkaline. Small changes in pH can affect the association process at the active site between enzyme and substrate. The attraction between substrate and enzyme is often the result of small electric charges at the active site, and these are disrupted by changes in H^+ ion concentration. Extremes of pH, i.e. very acid or alkaline conditions, cause permanent denaturation. Enzymes have an optimum pH for efficient functioning. Usually this is around pH7, the normal pH of cells. Notable exceptions are the enzyme pepsin, which is found in the stomach of vertebrates and works best in highly acidic conditions in the range pH 1-2, and the enzyme trypsin which is found in the duodenum and has an optimum pH of 9.

Temperature

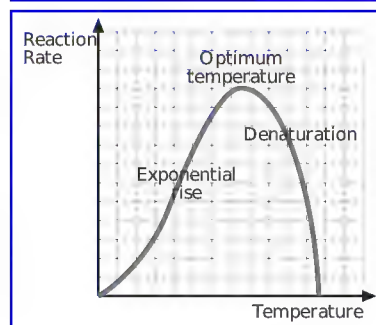
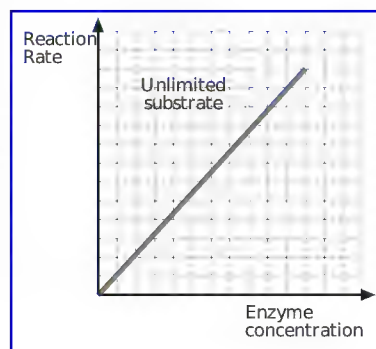
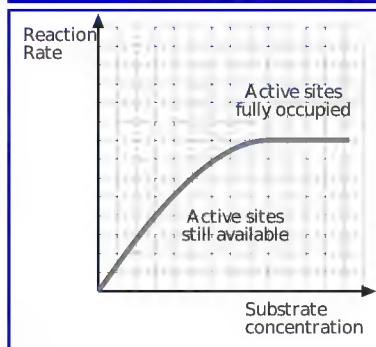
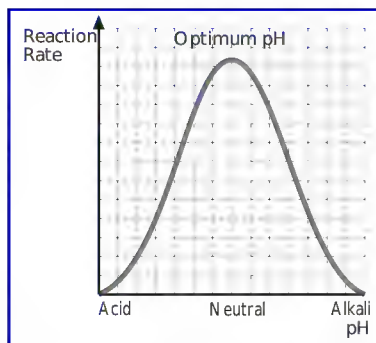
Temperature affects the rate of all chemical reactions. As the temperature increases, so does the kinetic energy of the reacting molecules. More enzyme/substrate complexes are formed as the reactants collide more frequently, and the reaction rate rises exponentially, doubling for every ten degree rise in temperature. However, enzymes, like all proteins, suffer irreversible alterations in molecular shape above certain temperatures as a result of the breaking of chemical bonds within the molecule, so that in enzyme-catalysed reactions, there is an **optimum** temperature above which the reaction rate drops sharply. For an enzyme, any change in the shape of the active site means a loss of function, the enzyme is said to be **denatured**. For most enzymes, the optimum temperature is around 40°C, but there are extreme exceptions. For example, some bacteria living in hot springs have enzymes which function at temperatures above 85°C, whilst the ice fish of the Antarctic possess enzymes which operate at -2°C.

Enzyme Concentration

Enzyme concentration exerts a direct effect on the rate of the reaction. As long as there is a plentiful supply of substrate, any increase in the number of enzyme molecules will result in a proportional increase in the number of enzyme-substrate complexes and therefore an increase in the rate of reaction.

Substrate concentration

Substrate concentration has a similar effect. At low substrate concentrations, not all the active sites will be occupied, so the rate of the reaction depends upon the concentration of substrate alone. As the concentration of substrate increases, so all of the available active sites will become filled. The upper limit being determined by the amount of enzyme molecules available.

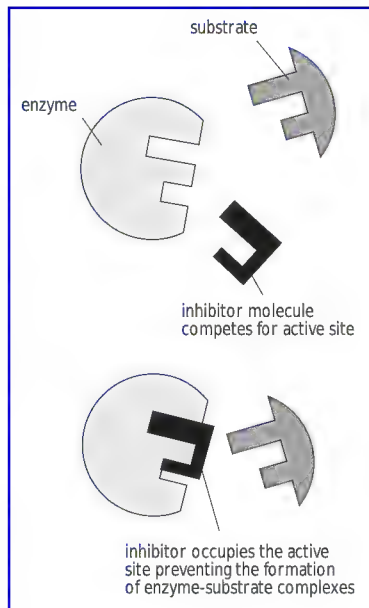


Enzyme Inhibitors

Enzyme inhibitors are substances which interfere with the active site of the enzyme. They do this either directly by blocking it, or indirectly by attaching to another part of the enzyme and altering its molecular shape so that the enzyme-substrate complex cannot be formed.

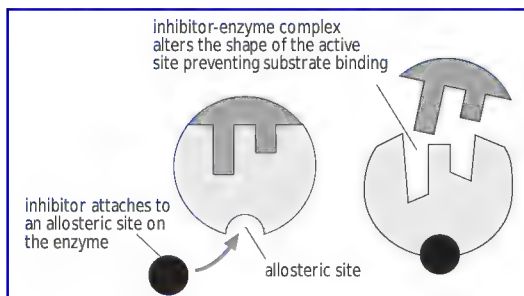
Competitive inhibitors

These are substances which have a molecular structure resembling that of the substrate. They attach themselves to the active sites forming inhibitor/enzyme complexes and cause the reaction to slow down or stop altogether. The effect of competitive inhibitors depends upon the relative concentrations of the substrate and inhibitor, and also the relative stability of the enzyme/substrate and enzyme/inhibitor complexes. This type of inhibition is seen in the action of sulphonamide drugs, which compete for the active site of a key bacterial enzyme involved in synthesising an essential growth factor, not found in the cells of the host.

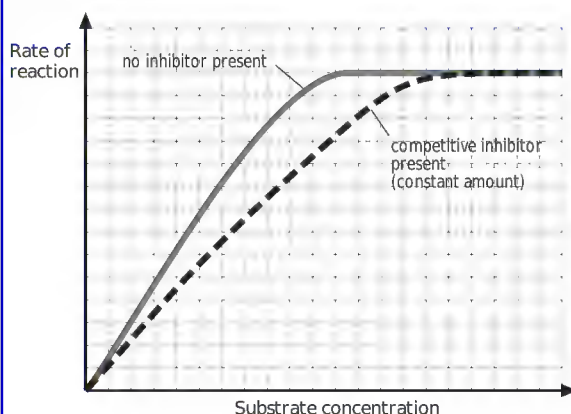


Non-competitive inhibitors

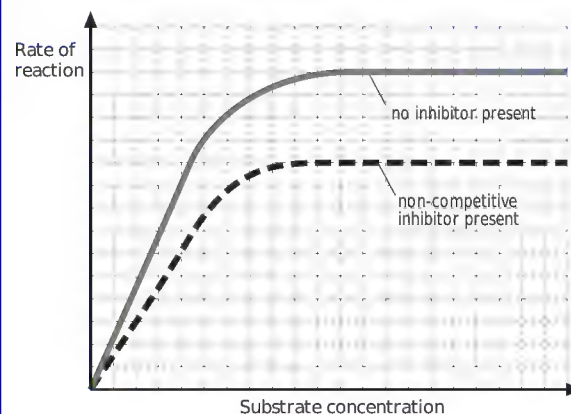
These are substances which bind onto the enzyme at a place other than the active site called an **allosteric site**, causing the enzyme to change shape sufficiently to stop enzyme-substrate complexes being formed. Non-competitive inhibitors are not similar to the substrate molecules, and are not affected by the substrate concentration. The ions of some heavy metals such as arsenic and cyanide may bind onto allosteric sites and act as poisons, stopping important enzyme controlled reactions. Some enzyme molecules possess an allosteric site which must be occupied by an **activator** substance to give the enzyme its correct working shape. Without the activator, the enzyme will not work. Inhibitors and activators are important in regulating the activity of enzymes.



The effect of a competitive inhibitor



The effect of a non-competitive inhibitor



◆ CHECKPOINT SUMMARY

- ◆ All enzymes are protein in nature, and fall into the group of 'globular' proteins with a complex 3D structure
- ◆ Enzymes are catalysts which speed up the rate of reaction of much larger amounts of reactants without themselves being used up in the process
- ◆ Unique amongst catalysts they are denatured by extreme conditions of pH and temperature etc.
- ◆ Their activity depends upon their complex 3D shape which creates an active site which combines with the substrate to form an enzyme/substrate complex which lowers the activation energy of reactants
- ◆ Their complex 3D shape also accounts for enzyme specificity as the active site has a shape complementary to the shape of the substrate
- ◆ Disruption of the 3D shape accounts for denaturation
- ◆ pH has an effect on the 3D shape of enzymes, especially the active site which has an electric charge
- ◆ Any effect on the active site alters the reactivity of the enzyme. There is an optimum pH, either side of which reactivity drops
- ◆ Extremes of pH permanently disrupt the active site and thus denature the enzyme.
- ◆ Similar effects are seen with temperature, except low temperatures do not denature enzymes. The rate of enzyme catalysed reactions, like all chemical reactions, are reduced with low temperatures
- ◆ Optimum conditions of pH and temperature vary with the particular enzyme, which reflects the conditions in which the cell exists
- ◆ The relative amounts of enzyme and substrate affect the rate of reaction as it affects the formation of the enzyme substrate complex. Only one can be rate regulating or limiting at any one time.

1.6

Digestion

Contents

Extracellular digestion

- ▼ Extracellular digestion exemplified by a saprophytic fungus.
- ▼ The principles of the use of starch-agar plates for assaying carbohydrase activity.

Digestion in humans

- ▼ The generalised structure of the human gut wall.
- ▼ The generalised structure of the gut wall in the oesophagus, stomach, duodenum and ileum related to the functions of these organs.
- ▼ The sites of production and action of:
 - amylases;
 - endopeptidases;
 - exopeptidases;
 - lipase;
 - maltase;
 - bile.
- ▼ Mechanisms for the absorption of food by the ileum, including the roles of diffusion, facilitated diffusion and active transport.

Introduction

Digestion may be defined as the process by which large, insoluble food molecules are broken down into smaller soluble molecules by the action of enzymes. Digestion may occur inside or outside the body of living organisms. In mammals food is ingested (eaten) and then digested by enzymes in the gut lumen and inside cells of the intestinal epithelium.

In fungi, however, digestion occurs outside the body. Enzymes are secreted onto a food substrate and the products of digestion are absorbed. This type of digestion is known as extracellular digestion. (It should be noted that term extracellular digestion simply means 'digestion outside the cell' so the action of many enzymes in the mammalian gut may also be described as extracellular. In the context of saprophytes it is often taken to mean 'outside the body').

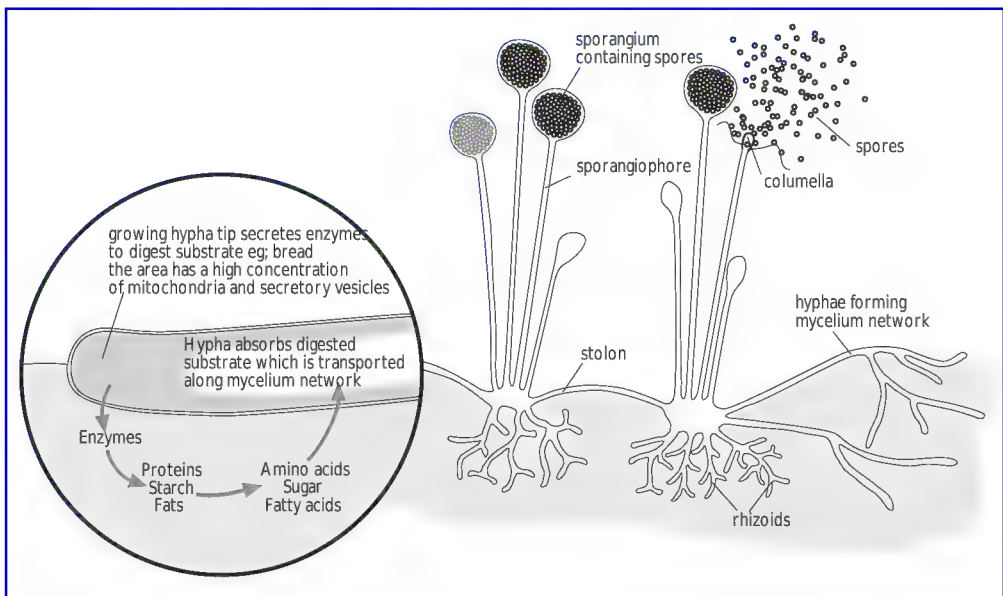
EXTRACELLULAR DIGESTION

Extracellular digestion in *Rhizopus* a saprophytic fungus

Rhizopus is a common form of fungus known as a pin mould which lives on dead organic matter, a mode of life known as saprophytic (saprobiontic). It is often seen growing on bread or similar substances. The visible structures are the small pin shaped sporangia which produce spores. The body (mycelium) is made up of microscopic threads known as hyphae, which are inside the substrate.

The tips of the hyphae secrete digestive enzymes to digest the large molecules of which the bread is made. These include carbohydrase enzymes (to break down carbohydrates such as starch to glucose), proteases (to break down proteins to amino acids) and some lipases to break down fats to fatty acids and glycerol. The soluble products of digestion (simple sugars, amino acids, fatty acids and glycerol) are then absorbed by the hyphae which have a large total surface area to facilitate this process.

As a result of the large surface area to volume ratio, the absorbed molecules are rapidly transported to all parts of the mycelium by diffusion, where they are used in the metabolism and growth of the fungus. It should be noted that such extracellular digestion requires both water and warmth. This explains why moulds will not grow on dry food but will appear within a short space of time if bread is moist and warm (enclosed in a plastic bag, for example). The spores are present everywhere, awaiting suitable conditions for germination.



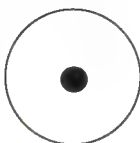
The principles of the use of starch-agar plates for assaying carbohydrase activity

Agar is a jelly like substance made from complex polysaccharides derived from a type of red algae (seaweed). It is commonly used to culture bacteria, but can also be a useful tool in assessing the activity of carbohydrase enzymes. To achieve this the following procedure is followed:

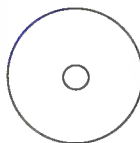
- ▼ Agar is mixed with starch and left to set in a petri dish.
- ▼ Several wells of equal size are cut in the agar using a cork borer or similar tool.
- ▼ Solutions of carbohydrase enzymes (e.g. amylase) or substances containing carbohydrase enzymes such as germinating seeds are placed in each well.
- ▼ The plate is covered and incubated at a suitable temperature for several hours.
- ▼ During the incubation time the carbohydrase enzyme diffuses out from the wells into the surrounding starch-agar mixture and digests the starch.
- ▼ Iodine solution is added to the starch-agar mixture which results in a uniform blue-black colouration wherever starch remains undigested.
- ▼ Where the enzyme has digested the starch to maltose the agar remains clear, these zones are circular as the enzyme diffuses out from the well at an equal rate in all directions.
- ▼ The size of such clear zones can be measured and is proportional to the amount of enzyme diffusing out from the well, which in turn is proportional to the amount of enzyme in the original sample.
- ▼ Comparisons can also be made between enzymes incubated at different temperatures, and between enzymes from different sources.
- ▼ In these ways carbohydrase activity can be assayed (assessed).

Starch-agar plates assaying carbohydrase activity

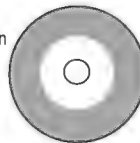
Methylene
Blue



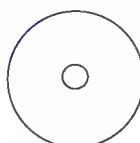
5%
Amylase
solution



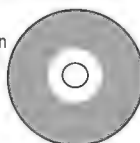
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



2.5%
Amylase
solution



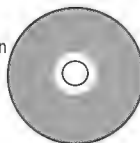
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



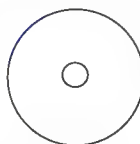
1%
Amylase
solution



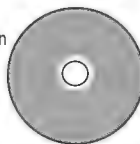
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



0.5%
Amylase
solution



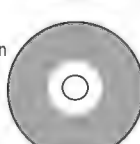
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



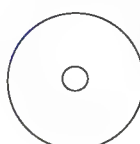
Unknown
Concentration
Amylase
solution



Plates flooded with Iodine in
potassium iodide solution
after 60 minutes
(in this case the unknown
was 2.5% amylase solution)



Boiled
Control



Plates flooded with Iodine in
potassium iodide solution
after 60 minutes

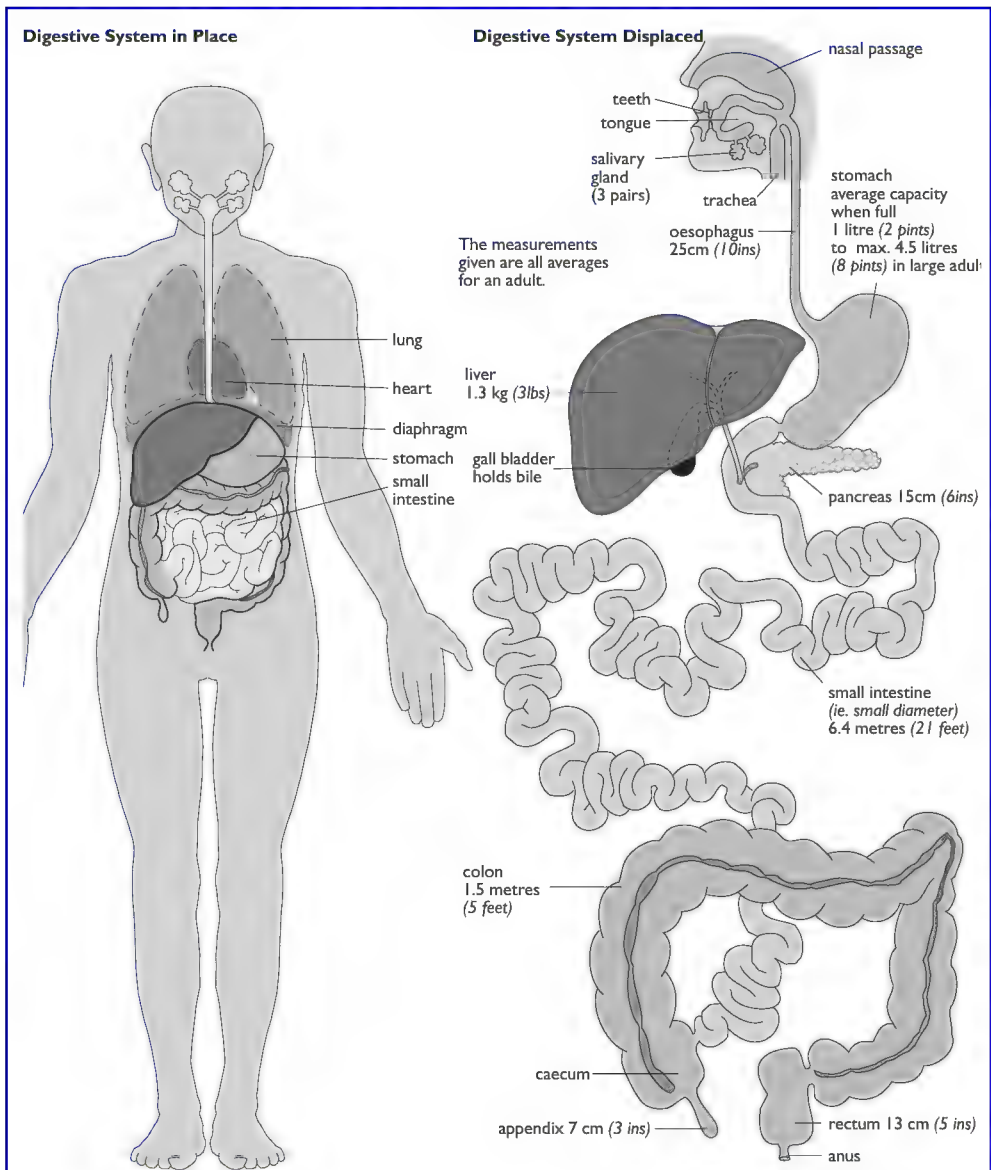


◆ CHECKPOINT SUMMARY

- ◆ Digestion is the breaking down of larger food molecules into smaller absorbable molecules by the action of enzymes
- ◆ Digestion may occur outside (extracellular digestion) or inside (intracellular) the body of living organisms
- ◆ Fungi secrete digestive enzymes onto a food substrate and the products of digestion are absorbed by diffusion
- ◆ It should be noted that term extracellular digestion simply means 'digestion outside the cell' so the action of many enzymes in the mammalian gut may also be described as extracellular. In the context of fungi it is often taken to mean 'outside the body' rather than just the cells
- ◆ Starch agar plates can be used to assess the activity of (assay) carbohydrase enzymes
- ◆ Carbohydrase enzymes (e.g. amylase) or substances containing carbohydrase enzymes such as germinating seeds are placed in each well
- ◆ Carbohydrase enzymes diffuse from the wells into the surrounding starch-agar mixture and begin to digest the starch
- ◆ The plate is then flooded with iodine in KI solution and the size of clear zones after a given time period can be used as a measure of the rate of enzyme catalysed reaction.

DIGESTION IN HUMANS

In humans, as in all other mammals, digestion takes place within a highly specialised alimentary canal or gut. This structure which forms a continuous tube from mouth to anus is divided into distinct regions based on differences in appearance and function. The main regions of the gut are, the buccal cavity (mouth), oesophagus, stomach, small intestine (duodenum, jejunum and ileum), and large intestine (caecum and appendix, colon, and rectum).



The generalised structure of the human gut wall.

From the oesophagus to rectum the wall of the gut is comprised of four layers: the innermost mucosa, submucosa, muscularis externa and outermost serosa. These layers, particularly the mucosa and submucosa are modified in each region according to their functions.

Note that from the last third of the oesophagus on, all the muscle in the gut wall is involuntary (smooth, unstriated) muscle, which is under reflex control of the autonomic nervous system and hormones, and not under voluntary control.

Mucosa. This innermost layer lining the gut consists of epithelial cells overlying connective tissue and a thin layer of muscle. The epithelial cells have a range of secretory and absorptive functions in different gut regions. In all regions the mucosa contains numerous lymphocytes (white blood cells) to help protect from harmful foreign substances.

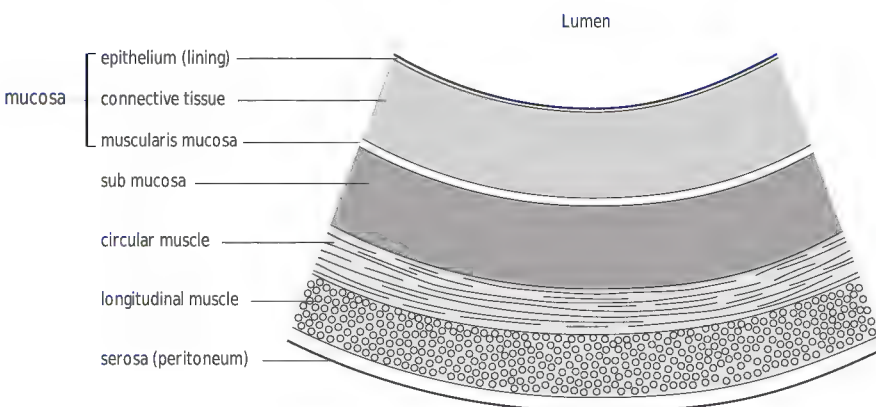
Submucosa. This consists of a layer of dense connective tissue containing large blood vessels which send branches into the mucosa and muscle layers. Lymphatic vessels and nerve fibres are also present.

Muscularis externa. This consists in most regions of an outer longitudinal muscle layer and an inner circular muscle layer. Alternating waves of contraction and relaxation in these (peristalsis) move food through the gut. Nerve fibres run through the muscle layers to control the movements of the muscles, although the muscle itself is capable of contracting without nervous stimulation (myogenic).

Serosa. This layer of squamous epithelial cells forms a membrane which covers the outer surface of the gut wall.

In addition to these four layers, the gut region also contains numerous glands secreting enzymes, mucus and other substances into the gut lumen. Some of these glands are contained in the mucosa and submucosa layers whilst others are found outside the gut e.g. the pancreas.

General plan of tissues of alimentary canal



Oesophagus

The function of the oesophagus is to convey food from the buccal cavity (mouth) to the stomach. Since the foods may contain hard or sharp pieces, the mucosa has a tougher form of epithelium known as stratified epithelium. This is several layers of cells thick and protects underlying structures from damage. Mucous glands in the mucosa and submucosa secrete mucus to lubricate the food. There is a thick muscularis externa in this region again because swallowed food may be in large pieces and need strong waves of contractions to move down to the stomach.

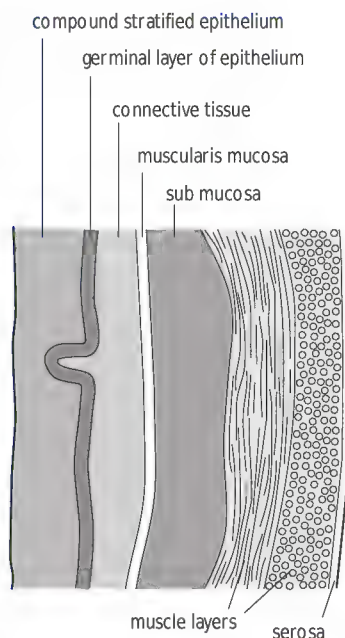
Stomach

The stomach receives swallowed food, which is mixed with gastric juice containing mucus, water, the protein digesting enzyme pepsin, and hydrochloric acid. The acid sterilises the food, and activates and provides optimum conditions for pepsin to start the digestion of proteins. The acid conditions inhibit the action of the salivary amylase on starch. The food mixture now known as chyme is temporarily stored in the stomach, whilst small amounts are released into the duodenum of the small intestine. Some water may be absorbed in the stomach.

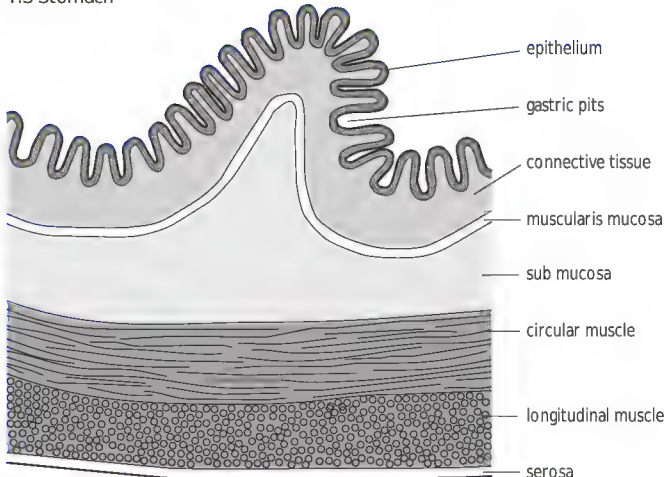
The mucosa of the stomach is folded and contains gastric glands which open into gastric pits in the surface. There are three types of secretory cells in these glands: mucous secreting cells, oxyntic cells secreting hydrochloric acid and chief cells secreting pepsinogen which is converted into the active enzyme pepsin by the low pH of the stomach contents. The digestion of proteins by the enzyme pepsin begins here. Pyloric glands near to the pyloric sphincter secrete a more alkaline mucus.

The muscularis externa is crucial for three stomach functions: receiving food, mixing it and moving it on by peristalsis. There are three muscle layers (longitudinal, circular and oblique) which are capable of producing these churning and mixing movements. Nerves within the muscle layers send information to the brain about the fullness of the stomach which helps to control appetite. Nervous and hormonal mechanisms control the flow of gastric juice.

T.S Oesophagus



T.S Stomach



Oblique muscle is not visible

Small intestine

The small intestine is divisible into three regions, the duodenum, jejunum and ileum, and is where the majority of digestion and absorption of digested food takes place.

The mucosa and submucosa of the small intestine are highly specialised for these functions. These layers form folds which increase the surface area for the absorption of the end products of digestion, and also slow the movement of food which increases the time available for digestion and absorption in this region..

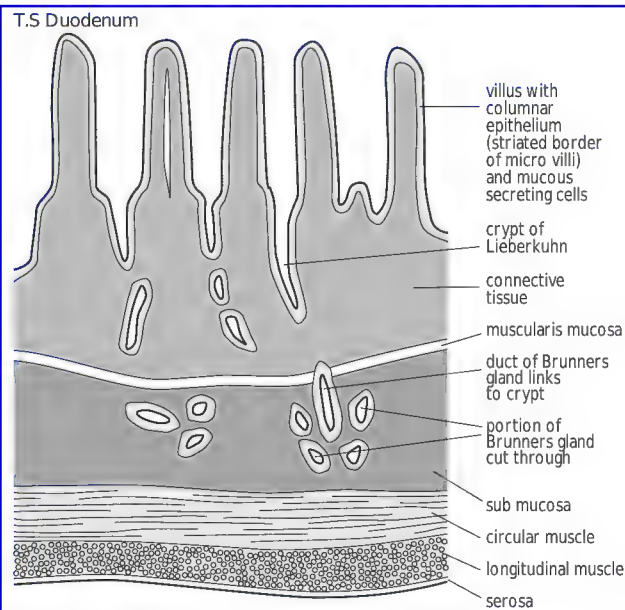
The surface area is further increased by finger-like and leaf-like villi formed from the mucosa.

Each villus contains blood capillaries to absorb small molecules such as glucose, and a lymphatic capillary (the lacteal) to absorb fats. The cells of the villus are also specialised with surface projections called microvilli, enzymes attached to the membranes to complete digestion, and goblet cells which secrete mucus. The villi also contain smooth muscle fibres, contractions of which help increase the exposure of the villi to the passing chyme.

In the mucosa, glands known as the crypts of Lieberkuhn, secrete alkaline intestinal juice to neutralise acid chyme from the stomach. Brunners glands in the sub mucosa of the duodenum secrete alkaline mucus which coats and protects the surface of the small intestine against acid chyme and stomach enzymes.

The muscularis externa in this region is responsible for 2 types of movement: mixing and circulating the chyme with intestinal juice in each part of the small intestine (known as segmentation or shuttling) and moving the chyme onwards by peristalsis towards the large intestine.

Completion of digestion and absorption of the end products of digestion occurs in the ileum.



CHECKPOINT SUMMARY

- ◆ Main regions of gut are; mouth, oesophagus, stomach, duodenum, ileum, colon, and rectum
- ◆ From the oesophagus to the rectum the wall of the gut is comprised of four layers: the innermost mucosa, submucosa, muscularis externa and outermost serosa. These layers are modified in each region in accordance with its function
- ◆ The oesophagus is lined with stratified epithelium. This is several layers of cells thick and protects underlying structures from damage. Mucous glands secrete mucus to lubricate the food, and thick layer of smooth muscle pushes food down by peristalsis
- ◆ The stomach wall is folded and contains gastric glands which open into gastric pits. There are three types of secretory cells in these glands: mucus secreting cells, oxyntic cells secreting hydrochloric acid and chief cells secreting pepsinogen (later converted to the pepsin)
- ◆ The smooth muscle of the walls contracts to mix and move food on into the small intestine
- ◆ The small intestine submucosa and mucosa form folds and villi which increase the surface area for the absorption of food and slow the movement of food
- ◆ Each villus contains blood capillaries to absorb all nutrients, and a lymphatic capillary (the lacteal) to absorb fats. The cells of the villus bear surface projections called microvilli.

Digestive enzymes of the human intestine

The digestion of food begins in the buccal cavity and stomach although the majority of digestion takes place in the small intestine where digested products are also absorbed. A number of different enzymes are involved, each specific to one type of substrate. Enzymes are produced in four main locations within the intestine :

- ▼ From glands within the mucosa and submucosa
- ▼ From large glands outside the gut wall
- ▼ On the surface of epithelial cells of the small intestine mucosa
- ▼ Inside epithelial cells of the small intestine mucosa

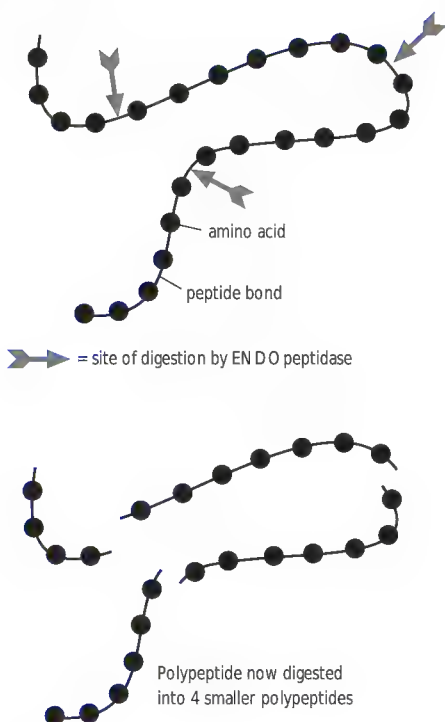
The table below shows the sites of production, sites of action and function of some of the main groups of enzymes, including bile (which does not contain enzymes). It should be noted that many of the enzymes (for example pepsin and trypsin) are released as inactive precursors and are only activated when they reach their site of action. Other enzymes may be involved in this activation process. Hormones, interacting with signals produced by the presence of food in the gut also play a very significant role in determining the timing of enzyme release.

Enzyme	Site of production	Site of action	Function
Amylase I	Mouth (salivary amylase)	Mouth	Digests starch to maltose
II	Pancreas (pancreatic amylase)	Lumen of small intestine	Digests starch to maltose
Endopeptidase I	Stomach (pepsin)	Stomach	Digests proteins to polypeptides and large polypeptides to smaller polypeptides (hydrolyses internal peptide bonds within proteins or polypeptides)
II	Pancreas (trypsin)	Lumen of small intestine	
Exopeptidase I	Epithelial cells of ileum	Surface of epithelial cells of ileum	Digests polypeptides and peptides to amino acids (hydrolyses terminal peptide bonds to remove amino acids)
II	Pancreas	Lumen of small intestine	
Lipase	Pancreas	Lumen of small intestine	Digests fats to fatty acids and glycerol
Maltase	Epithelial cells of ileum	Surface of epithelial cells of ileum	Digests maltose to glucose

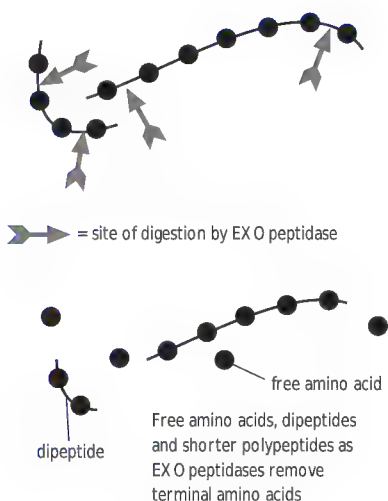
Digestive secretions which are not enzymes

Bile	Liver (stored in gall bladder)	Small intestine	Emulsifies fats, so increasing surface area for action of lipase
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Action of Endopeptidases



Action of Exopeptidases



◆ CHECKPOINT SUMMARY

- ◆ Enzymes are produced in four main locations within the intestine
- ◆ From glands within the mucosa and submucosa
- ◆ From large glands outside the gut wall
- ◆ On the surface of epithelial cells of the small intestine mucosa
- ◆ Inside epithelial cells of the small intestine mucosa
- ◆ The sites of production and action of amylases, endopeptidases, exopeptidases, lipase, maltase, and bile ensure digestion before the end of the small intestine
- ◆ The action of salivary amylase continues for a short time in the stomach but is denatured by the stomach acid
- ◆ Endopeptidases hydrolyse peptide bonds within proteins producing smaller polypeptides, and are secreted by the stomach and pancreas
- ◆ Exopeptidases hydrolyse terminal peptide bonds at the ends of proteins releasing amino acids, and are secreted by the cells of the ileum and pancreas
- ◆ Lipase digests lipids and is secreted by the pancreas
- ◆ Bile contains no enzymes but provides alkaline conditions and salts that emulsify fat, and is indispensable to the digestive process
- ◆ Maltase digests maltose to glucose, and is located in the cell membrane of the cells of the ileum
- ◆ The end products of digestion enter the epithelial cells of the ileum by simple diffusion, facilitated diffusion, active transport and endocytosis
- ◆ Diffusion is the movement of substances from a high concentration to a lower concentration. No energy and no carrier molecules are required
- ◆ Facilitated diffusion is the movement of substances from a high concentration to a lower concentration using specific protein carrier molecules or protein channels, no energy is required
- ◆ Active transport is the movement of substances against their concentration gradient (from low to high) via protein carrier molecules. Energy in the form of ATP from respiration is required.

Mechanisms for the absorption of products of digestion by the ileum.

Large food molecules digested by the enzymes noted above need to pass from the ileum into the blood capillaries and lymphatic capillaries for transport around the body and distribution to appropriate tissues. To do this they must first enter the epithelial cells of the ileum and then leave them with or without further processing. There are several mechanisms by which this process is achieved which reflect the physical and chemical characteristics of the digested molecules. The mechanisms are simple diffusion, facilitated diffusion, active transport and endocytosis. Basic features of these mechanisms are:

Diffusion: the movement of substances from a high concentration to a lower concentration. No energy and no carrier molecules required.

Facilitated diffusion: the movement of substances from a high concentration to a lower concentration using specific protein carrier molecules or protein channels in the cell surface membrane. No energy required.

Active transport: the movement of substances against their concentration gradient (from low to high) via protein carrier molecules in the cell surface membrane. Energy in the form of ATP is required.

Endocytosis: substances are taken up into vesicles produced by enclosures of the cell surface membrane.

Glucose and other monosaccharides

Owing to their relatively large size and the fact that they are not lipid soluble, these molecules are transported into the epithelial cells by facilitated diffusion (fructose), and active transport (glucose and galactose). Each monosaccharide will have a carrier molecule of a specific complementary shape. Once inside the epithelial cell the monosaccharides are moved out into the capillaries by facilitated diffusion.

Amino acids

These are taken into epithelial cells of the ileum by active transport. Different types of amino acids (including acid, basic, neutral) have different carrier proteins responsible for their transport. They leave by facilitated diffusion.

Fatty acids and glycerol

These products of fat digestion move into epithelial cells by simple diffusion. This is possible because they are both small and lipid soluble so dissolve in the cell membrane. Once inside the epithelial cells they are reassembled into triglycerides (one glycerol and three fatty acids), and formed into tiny droplets called chylomicrons. These are then coated in proteins and are moved from the cells to the lacteals by exocytosis using Golgi vesicles.

Water, alcohol and other small molecules

Owing to their small size these are absorbed into the epithelial cells by simple diffusion. These molecules also move from epithelial cells to capillaries by simple diffusion.

2 Genes and Genetic Engineering

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2.1

The Genetic Code

Content:

The gene

- ▣ Genes as sections of DNA which contain coded information that determines the nature and development of organisms
- ▣ Genes exist in different forms called alleles which are positioned in the same relative position (locus) on homologous chromosomes.

Structure of DNA

- ▣ DNA is a stable polynucleotide
- ▣ The double helix structure of DNA: the components of DNA nucleotides; the sugar-phosphate backbone; specific base pairing and hydrogen bonding between polynucleotide strands.

Replication of DNA

- ▣ The semi-conservative mechanism of DNA replication, including the role of DNA polymerase.

The genetic code

- ▣ How DNA acts as a genetic code by controlling the sequence of amino acids in a polypeptide.
- ▣ Codons for amino acids are triplets of nucleotide bases.

Role of nucleic acids in protein and enzyme synthesis

- ▼ The structure of RNA.
- ▼ The production of mRNA in transcription and the role of RNA polymerase.
- ▼ The roles of ribosomes, mRNA and its codons, and tRNA and its anticodons in translation.

Mutation

- ▼ New forms of alleles arise from changes (mutations) in existing alleles.
- ▼ Gene mutation as the result of a change in the sequence of bases in DNA, to include addition, deletion and substitution.
- ▼ Mutations occur naturally at random. High energy radiation, high energy particles and some chemicals are mutagenic agents.

THE GENE

DNA carries the genetic code by which hereditary information is passed from generation to generation via the nuclei of the gametes, and from cell to cell via the nuclei of the cells produced by division of the fertilized egg. Genes are sections of DNA which contain coded information that determines the nature and development of organisms. A single **gene** is a length of DNA which codes for the formation of a single polypeptide, containing the specific instructions for the number and arrangement of each amino acid in the polypeptide chain from which the protein is constructed.

In a non-dividing cell, the nucleus contains scattered '**chromatin**' material which is a mixture of DNA and associated proteins called histones. When the DNA is in this dispersed form, it is genetically active, sending out information to the cytoplasm to control the complex cell processes. When the cell is about to divide the chromatin concentrates or condenses into an inactive state to form the **chromosomes**. In this state the DNA is more resistant to damage during the processes of nuclear and cell division. Moreover, since it is in discrete bodies like the chromosomes, the division of the genetic material between the nuclei can be carefully controlled, reducing the danger of any being lost. It is only in this condensed form that chromosomes are ever visible in the nucleus when stained and viewed under the light microscope.

The nucleus of each cell of a diploid organism contains two sets of chromosomes; one maternal from the female gamete and one paternal from the male gamete. As a result, each chromosome has a 'partner' in the other set which carries genes for the same characteristics. Two such chromosomes are said to form a **homologous pair**. A gene is a specific length of DNA occupying a specific position called a **locus** (plural loci). Therefore homologous chromosomes have matching gene loci. The genes on the homologous partner may either be identical, or mutations could have occurred to produce altered forms of the gene. Different forms of a specific gene are known as **alleles**, and a pair are known as an **allelomorphic pair**.

Structure of DNA

DNA (Deoxyribo(se)nucleic Acid) is a polymer made up of repeating sub-units of **nucleotides**, it is therefore known as a **polynucleotide**. What makes DNA so important and so unique is that it contains within its structure the genetic code and it can copy itself exactly. Crucial to this ability is the stability of DNA's molecular structure, it is a stable polynucleotide.

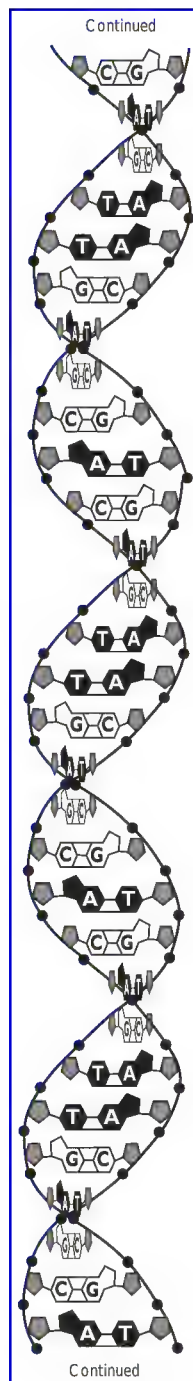
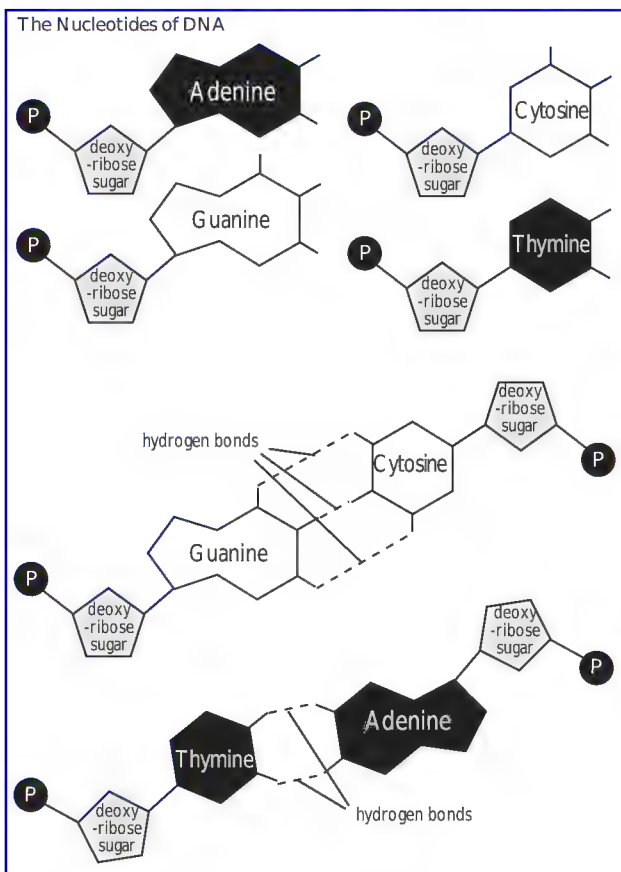
The Double Helix

The nucleotides which make up DNA consist of three main parts: a **pentose (5 carbon) sugar** joined to a **phosphate group** and a nitrogen-containing **organic base**. There are four different types of nucleotide in DNA each with a different organic base. The bases are: **adenine** (A) and **guanine** (G) which belong to a group of chemicals called **purines**; and

cytosine (C) and **thymine** (T) which belong to a group called **pyrimidines**.

Chains of nucleotides link up by condensation reactions to form a spine made up of sugar and phosphate molecules with the bases sticking out to one side.

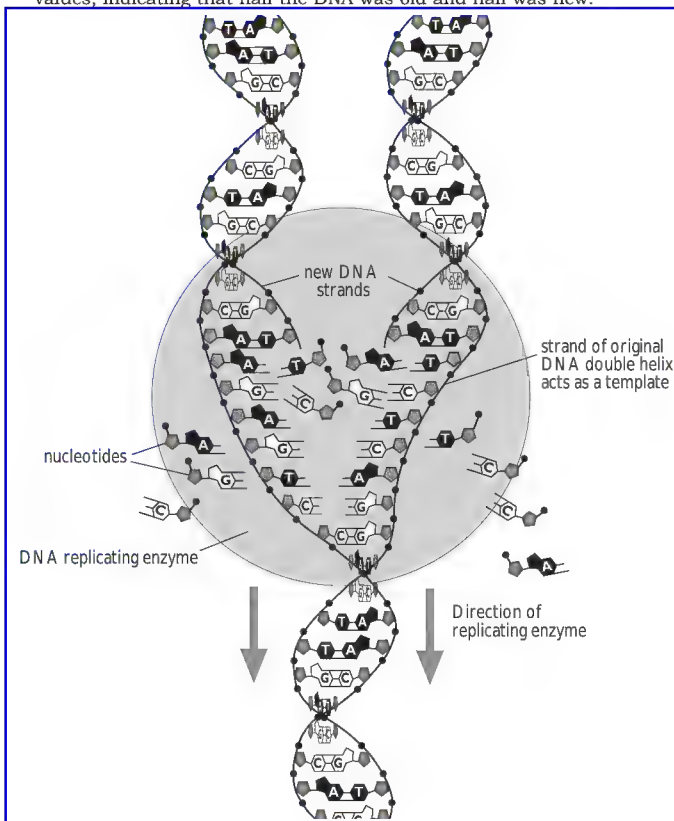
DNA consists of two such chains (**strands**) running in opposite directions to each other, lying side by side and linked by hydrogen bonds which form between adjacent bases. As the hydrogen bonds form, so the two strands of the DNA molecule twist into the spiral structure commonly known as the **double helix**. What determines the very special properties of DNA is the fact that **base pairing** occurs, that is, hydrogen bonds form only between specific pairs, namely A with T, and C with G. The strands are therefore **complementary** to each other. The sequence along one strand determines the sequence of the other (complementary) strand.



Replication of DNA

As the two strands of DNA are complementary, one strand can act as a pattern (**template**) for the formation of the other. What is needed to make an exact copy (replica) of the original is an assortment of the four different nucleotides, energy in the form of ATP, and an enzyme called **DNA polymerase**. The two original strands unwind, and each acts as a template for the construction of a new complementary strand. In this way two new double stranded molecules are synthesised, each with one original strand and one with a new strand. For this reason, the process of replication is said to be **semi-conservative**.

Experimental evidence for the semi-conservative mechanism of DNA replication includes experiments in which the DNA content of *E. coli*, grown on different nutrient media was weighed and compared. One batch of microorganisms was cultured on a medium containing a 'heavy' isotope of nitrogen (^{15}N), the DNA was extracted and weighed, then compared with that of organisms cultured on a medium containing normal (light) nitrogen (^{14}N). The *E. coli* cells grown on heavy nitrogen were then transferred to a new medium containing only light nitrogen and allowed to divide once to produce a new generation. The DNA of these daughter cells was extracted and weighed. It was found to be halfway between the heavy and light values, indicating that half the DNA was old and half was new.



The genetic code

The DNA base sequence forms a four letter language which, as you have seen, can be copied exactly every time a cell divides. This DNA base sequence determines the structure of all the proteins made by living cells, including the enzymes, structural proteins such as collagen, and membrane proteins. In other words, the base code determines the structure, functions and individuality of every organism. It does so by the sequence of bases along the DNA molecule determining the sequence of amino acids (primary structure) of a polypeptide, which gives each protein its particular characteristic.

Remember that up to twenty different types of amino acids may occur in a single protein. The DNA code has a four letter (A, G, C, T) base language. A triplet of bases (sequence of three in a row) codes for each amino acid, for example 'CGC' codes for the amino acid alanine (Ala), whilst 'GCT' codes for arginine (Arg), so the code 'CGC-GCT-CGC' would be translated as 'Ala-Arg-Ala' in a polypeptide. Note that the code is non-overlapping, i.e. it must be read 'CGC-GCT-CGC', not 'CGC-GCG-CGC-GCT...' etc.

Sixty four different triplet codes (**codons**) are possible with this four letter base language, more than sufficient to account for the twenty amino acids. In fact most amino acids are coded for by more than one codon, and the code is therefore said to be 'redundant'. The process of translating this code into the reality of a sequence of amino acids in a polypeptide chain, that is, the process of protein synthesis, involves another nucleic acid called **ribonucleic acid (RNA)**.

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA, GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA, AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

◆ CHECKPOINT SUMMARY

- ◆ The sequence of nucleotide bases on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living organisms.
- ◆ Three bases (a triplet codon) code for one amino acid.
- ◆ There are 64 (4^3) possible combinations of any three from four bases, but only 20 amino acids to be coded for.
- ◆ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.
- ◆ A gene is a sequence of nucleotide bases of a DNA molecule which codes for a polypeptide.
- ◆ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ◆ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.
- ◆ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood.

The structure of RNA

RNA (Ribo(se)nucleic acid), like DNA is a polynucleotide made up of four types of repeating nucleotides. It differs from DNA in the following three ways.

- ▼ RNA is a single strand
- ▼ The pentose sugar in RNA is ribose, not deoxyribose (molecules of ribose contain an additional oxygen atom)
- ▼ The pyrimidine base **Uracil (U)** is found in RNA, not Thymine (T) as in DNA.

Living cells produce three different types of RNA in their nuclei, each designed to carry out a specific function in protein synthesis. The three types are:

Messenger RNA (mRNA) consists of a single unfolded strand, between 75 and 3000 nucleotides long, produced in the nucleus as an exact copy of a selected section of the DNA code. mRNA molecules travel out of the nucleus to the ribosomes where proteins are assembled.

Transfer RNA (tRNA) molecules are shorter lengths of RNA, between 75 and 90 nucleotides long, twisted into a characteristic shape which allows one end to attach to a specific amino acid, and the other to expose a triplet of bases called the **anti-codon** which attaches to a codon on the mRNA at the site of protein synthesis. tRNA molecules bring amino acids to their correct position as the proteins are being assembled. Each amino acid is carried by a different tRNA molecule with a specific anti-codon.

Ribosomal RNA (rRNA) is manufactured within the nucleolus. Ribosomes are composed of an approximately 50:50 mixture of proteins and large rRNA molecules each consisting of more than 1000 nucleotides.

The manufacture of proteins (protein synthesis) involves the assembly of amino acids in a specific sequence, predetermined by the genetic DNA code. It is brought about by all three of the RNA types described above working in combination with each other, and is achieved in two stages.

- ▼ As the DNA carrying the genetic code does not leave the nucleus, the code must be copied into mRNA in the process called **transcription**.
- ▼ The mRNA containing a copy of the length of DNA code (gene) leaves the nucleus and associates with ribosomes. Then, in a process called **translation** involving tRNAs, the code is translated into a sequence of amino acids in a polypeptide.

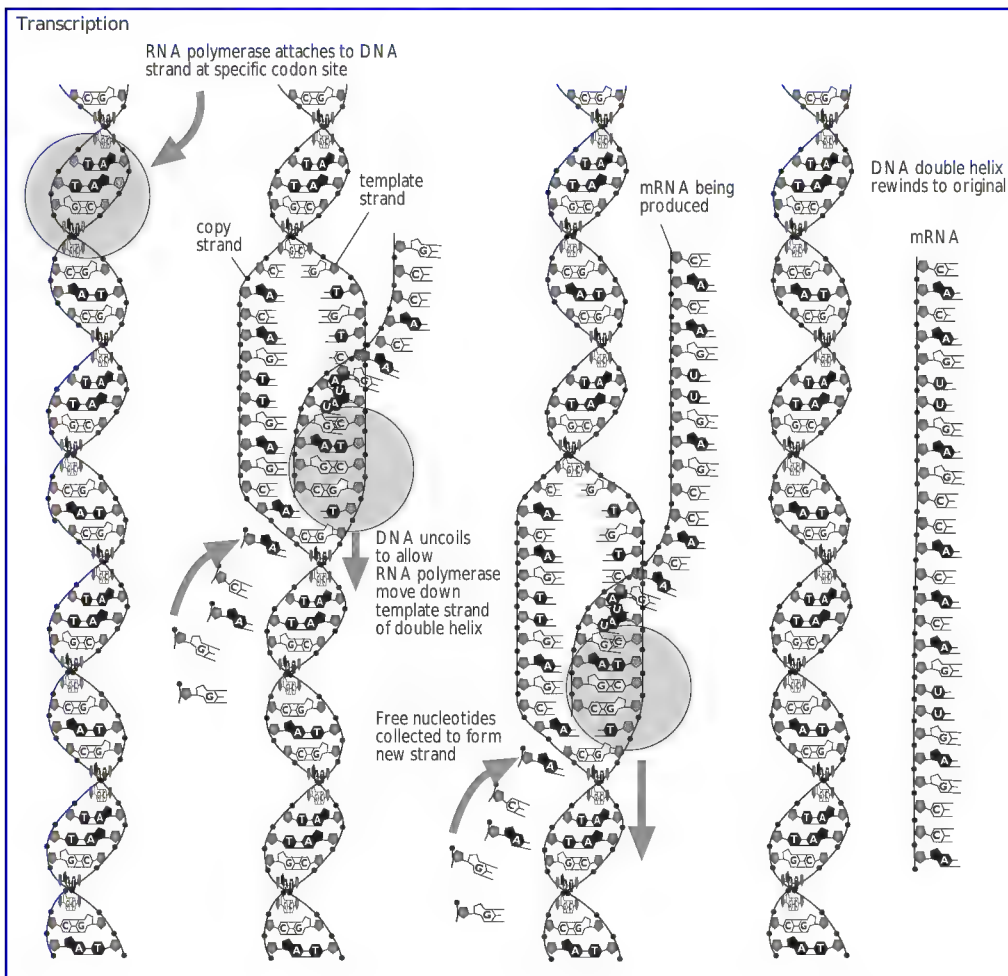
Transcription

Transcription begins as an enzyme called **RNA polymerase** attaches to the start codon of a gene. The DNA base sequence of this start codon is always TAC'. As it attaches, so the hydrogen bonds break apart to expose the base code. The enzyme then travels rapidly down the length of the gene like a zip fastener unzipping the DNA as it goes, and as it does so, a molecule of mRNA is formed copying one section (gene) of one of the DNA strands.

mRNA transcribes only one of the two DNA strands, called the **template strand**. mRNA is therefore an exact replica (except that 'U'

occurs instead of 'T') of the other strand, consequently referred to as the **copy strand**. When the enzyme reaches the stop sequence in the gene, transcription ends, the enzyme is free to start the process again, and mRNA is released out through a pore in the nuclear membrane to the ribosomes where the next stage occurs.

A complicating feature of the process of transcription in eukaryotic cells is that genes occur in pieces. In other words, the length of DNA to be read by mRNA has, in addition to the sections which code for amino acids, other sections of 'junk code' which are not translated into protein. The 'junk' sequences are called **introns** (because they stay within the nucleus) and the parts to be read and translated are called **exons**. mRNA is transcribed for the whole length but, before it is released from the nucleus, the introns are cut out with the assistance of enzymes, leaving a single, unpolluted, strand of mRNA.



Translation

Ribosomes are made up of two sub-units, one large, the other small which come together only for the purpose of translating a length of mRNA. mRNA binds to the small sub-unit at its start codon (AUG). It is then joined by the large sub-unit, along with a molecule of tRNA which has the anti-codon 'UAC' and carries the amino acid methionine. As the three different RNA types come together, the interface between the small and large sub-unit of the ribosome forms two binding sites allowing two triplet codons be lined up side by side. The process of translation can now begin.

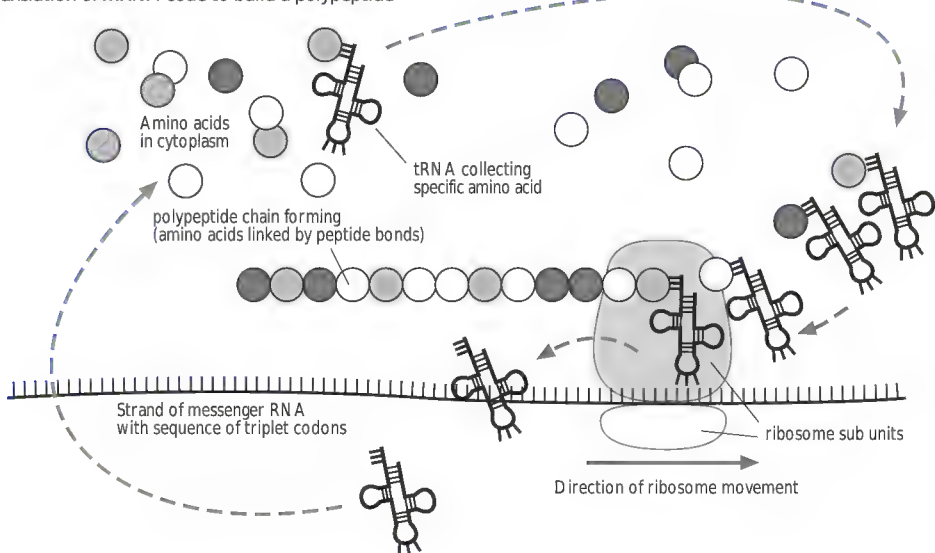
A second tRNA now occupies a position on the mRNA alongside the first and, as it does so, a peptide bond is formed between the two amino acids which have been carried in. The ribosome moves along the length of mRNA. As it does so, tRNA molecules that have completed their task are released to repeat the process, new tRNA molecules come to occupy the ribosomal coding sites, and the polypeptide chain grows ever longer. When the ribosome reaches the stop code (UAG) on the mRNA, it splits into its separate sub-units releasing the mRNA and the now complete polypeptide strand. A number of ribosomes move along the mRNA at the same time so that many polypeptide molecules are formed simultaneously. It is calculated that, in the average mammalian cell, more than a million new peptide bonds are being formed every second.

The polypeptide develops a secondary, tertiary, and sometimes a quaternary structure to become a functional protein, e.g. an enzyme. In this way the original DNA code has directed the synthesis of proteins, many of which are enzymes which catalyse further reactions in the cell.

◆ CHECKPOINT SUMMARY

- ◆ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.
- ◆ The genetic code (a gene) for a polypeptide is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.
- ◆ The ribosomes 'read' the genetic message on the mRNA, and tRNA delivers amino acids in the sequence determined by the code.
- ◆ There are 20 different types of tRNA, each specific to a particular amino acid.
- ◆ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.
- ◆ The amino acids combine by means of peptide bonds to give a polypeptide chain.
- ◆ The polypeptides subsequently form proteins.
- ◆ As enzymes are proteins their synthesis is controlled by DNA.

Translation of mRNA code to build a polypeptide



Mutation

If DNA can be regarded as the blueprint or recipe for building the body of a living organism, the site workers are the enzymes. DNA codes for specific enzymes which, in turn, take on the function of constructing other biomolecules from the raw materials taken in by nutrition. The actual appearance of an organism (its **phenotype**) is thus dependent upon the presence or absence of a particular enzyme. The smallest change (**mutation**) in the DNA code, even as small as an alteration in a single base, can disrupt the structure and function of an enzyme, with the result that a change occurs in the phenotype.

Mutation may be defined as 'any change in the structure or amount of DNA in an organism'. Mutations can be a change in individual genes, a change in chromosomal structure, or a change in chromosome number. Most mutations are harmful, or at least of no immediate advantage, as the organism is already adapted to its environment, and any change would tend to upset this adaptation. A few are beneficial, giving an advantage to the new variant organism, for example the acquisition of resistance to disease.

Most mutations occur in body cells (somatic mutations), in which case any effect they exert is restricted to the descendants of the original cell in which the mutation occurred. This can give rise to patches of mutated cells or mosaics. If the somatic mutation occurs in an immature bud in plants, the whole of the resultant branch will be mutant. The nectarine was one such 'bud sport' of a peach tree.

Mutations can also occur in the gamete-producing tissues, in which case the mutation will pass to all of the cells of the offspring via the fertilised egg. Once a mutation occurs it is perpetuated by further DNA replications. The altered gene is an allele of the original, and it is by this process that all new alleles arise. Dominant gene mutations express themselves immediately, whereas recessive mutations may be masked or hidden for several generations before expressing themselves.

Types of gene mutation

As has been seen, gene mutation is a change in the sequence of DNA bases which code for a particular polypeptide. Such a change might occur at any time in the complex series of events in DNA replication.

Deletions occur when one or more nucleotides are missing, resulting in the loss of one or more bases from the triplet codons.

Additions occur when one or more extra nucleotides are added, resulting in the gain of extra bases in the genetic code.

Substitutions occur when one base is substituted by another, altering the particular triplet codon..

The effect of a single base substitution is dramatically shown by the case of **sickle cell anaemia**. In this condition, newly-formed red blood cells are normal in shape but, when the oxygen in their haemoglobin is released to the body tissues, most of them change to an abnormal sickle shape. These sickle cells are destroyed in the spleen, thereby reducing the number of red blood cells. This cuts down the amount of oxygen carried. Also, due to their shape and lower flexibility, they do not pass through capillaries easily, and so reduce the efficiency of circulation. Sickle-cell anaemia is usually fatal in childhood. In less severe cases, sickle red blood cells are only produced when the supply of oxygen is low, e.g. at high altitudes or when the need for oxygen increases (such as during exercise). People affected in this way are said to 'have the sickle-cell trait' or to be 'carriers' of the condition.

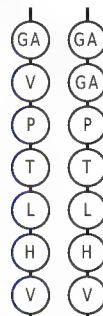
The sickle cell condition is caused by the replacement of just one base to produce the codon CAC instead of CTC. The result of this substitution is the insertion of the amino acid valine (coded for by CTC) instead of glutamic acid (coded for by CAC) into the polypeptide chain which makes up the molecule of haemoglobin. This single amino acid substitution is sufficient to cause a change in the tertiary structure of haemoglobin and affect the overall shape of red blood cells.

One normal copy of the gene is capable of producing sufficient normal haemoglobin, therefore the non-functioning allele is said to be recessive as the effect of possessing one copy is not seen under normal conditions. By the same argument, the normal allele is said to be dominant. It is only when two non-functioning alleles are present that the disease is seen.

The recessive sickle cell allele is rare in most populations (as would be expected with a semi-lethal allele i.e. one that is fatal when present in a double dose). However, in some parts of Africa, the sickle cell condition (trait) is found in as much as 40 per cent of the population. Its occurrence is related to the distribution of malaria, as the carriers of the trait are more resistant to the disease, survive and reproduce more, and therefore are said to have a selective advantage.

Other human diseases such as cystic fibrosis and phenylketonuria (PKU) are inherited as recessive traits.

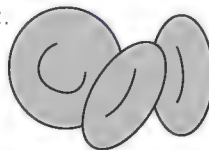
β chain of haemoglobin responsible for sickle cell anaemia



V - Valine
H - Histidine
L - Leucine

T - Threonine
P - Proline
GA - Glutamic acid

Normal R. B. C.



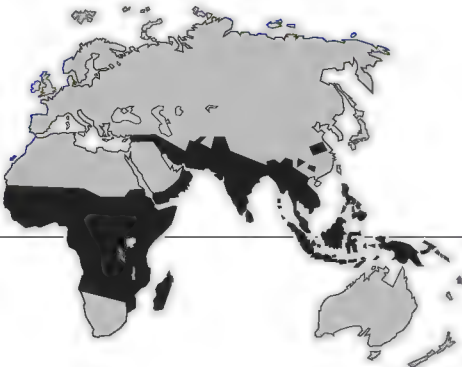
Sickle R. B. C.



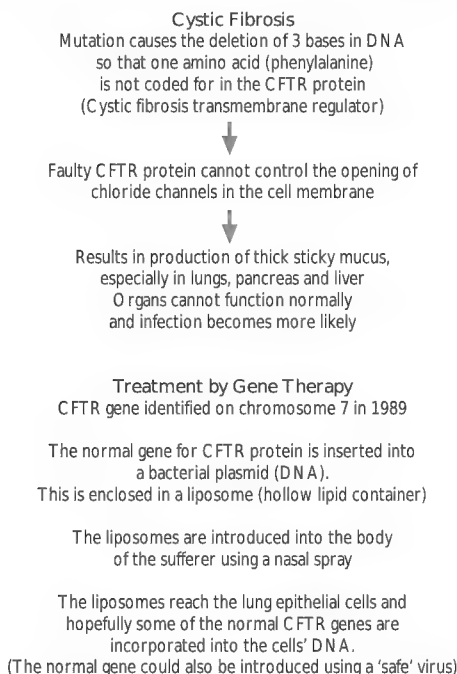
Distribution of sickle cell gene



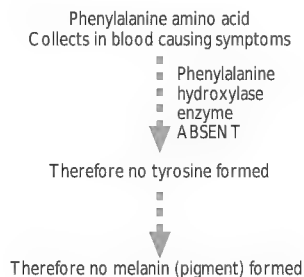
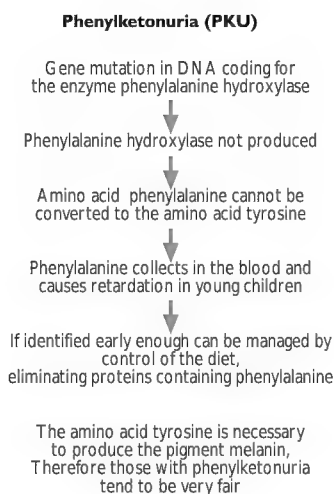
Distribution of malaria



Cystic fibrosis is one of the most common recessive genetic diseases and affects about 1 in 2000 babies born in the UK. It usually causes death by early adulthood. The disease is caused by a faulty protein known as cystic fibrosis transmembrane regulator (**CFTR**) which regulates transmembrane chloride ion channels in epithelial cells. A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced. This change in the primary structure of the protein alters its secondary and tertiary structures, preventing it from responding to signals to open chloride channels in cells. The overall effect of this is a build up of ions inside the cells which prevents water leaving by osmosis as it would normally do. Thus mucus released by cells is thicker than in normal individuals and sticks to cells causing inflammation and tissue damage, encouraging infections and preventing normal functioning of the organs concerned.



In the disease **phenylketonuria** (PKU) a gene mutation in the DNA coding for the enzyme phenylalanine hydroxylase means that this enzyme is not produced in the liver. Without phenylalanine hydroxylase the body cannot convert the amino acid phenylalanine to the amino acid tyrosine. This results in an accumulation of phenylalanine in the body which severely affects many systems. Untreated sufferers may be both mentally and physically retarded.



Mutagenic agents

DNA is a very stable polymer and, in nature, mutations occur naturally at random, but very rarely. The rate of mutation can be increased by subjecting an organism to a mutagenic agent (**mutagen**). Mutagens include various forms of high energy radiation such as X rays, gamma rays, and ultraviolet light; high energy particles such as α particles and β particles; and a number of chemical substances such as nitrosamines, benzene, constituents of tobacco smoke, and the breakdown products of some pesticides. Any change in the DNA caused by mutagens may lead to abnormal cell division resulting in cancer, so it is common for mutagens to also be carcinogens (cancer promoting substances) although not all carcinogens are mutagens.

Mutagens may affect the bases sequence of DNA or whole chromosome sets. Nitrous acid (HNO_2) causes changes in the molecular structure of bases. Some of which may not have any effect, for example, guanine may be converted to an alternative form called xanthine, but this pairs in the normal way with cytosine so no change occurs, but if adenine is converted to hypoxanthine, or cytosine to uracil, the bases fail to pair, the hydrogen bonding is disrupted and the molecular structure of DNA breaks down.

Some mutagens are used in gene technology to produce mutations deliberately. For example, the chemical mutagen colchicine inhibits the formation of the spindle in cell division (see 11.2) and it is used to double up the number of chromosomes in cells.

Ultra violet light affects pyrimidine bases, particularly thymine so that where adjacent thymine bases occur on the DNA chain, they link together causing the normal hydrogen bonding with adenine to break.

◆ CHECKPOINT SUMMARY

- ◆ A change (mutation) may occur in the sequence of bases in a gene, and this changed form of a gene is known as an allele of that gene.
- ◆ Mutations of a gene may occur many times in members of a species so there may be many alleles of a gene (multiple alleles) in a population, but each diploid organism can only ever have two alleles of a gene, at one particular locus on a pair of homologous chromosomes.
- ◆ The type of mutations that occur can include additions, deletions, and substitutions of bases, all of which alter the sequence of bases, and consequently the sequence of amino acids in the polypeptide chain for which the gene codes.
- ◆ Additions involve the inclusion of an additional base in the DNA sequence, which has a 'knock on' effect on all subsequent triplet codons.
- ◆ Deletions involve the loss of a base in the DNA sequence, which also has a 'knock on' effect on all subsequent triplet codons.
- ◆ Substitutions involve the replacement of a base by another; this type of mutation only affects one triplet codon, and consequently only one amino acid in the polypeptide chain (the fact that an amino acid is coded for by more than one triplet codon means that a substitution may not alter the amino acid sequence).
- ◆ Mutations occur naturally at random, but many factors (mutagenic agents) increase the rate of mutation.
- ◆ High energy radiation such as X rays, gamma rays, and ultra violet light cause ionisations in DNA molecules. The changed electrical charges alter the shape and therefore the function of DNA.
- ◆ High energy particles such as alpha particles, beta particles (electrons) and neutrons all damage DNA as they pass through the nucleus.
- ◆ Some chemicals such as benzene, xylene, nitrosamines, mustard gas, tar in tobacco smoke etc cause mutations.
- ◆ Many mutations lead to cancer; and agents that cause cancer are known as carcinogenic.
- ◆ Alterations in the polypeptide chain affect the secondary and tertiary structures of the final protein upon which its activity depends.
- ◆ A non-functioning enzyme can cause a metabolic block to occur in a metabolic pathway.

2.2

The Cell Cycle

Content:

Mitosis

- ▼ Mitosis as the replication of DNA in a parent cell which divides to produce two new cells, each containing an exact copy of the DNA of the parent cell.
- ▼ The stages of mitosis.

Applications of cloning

- ▼ The production of crops by vegetative propagation.
- ▼ The cloning of animals by splitting apart the cells of developing embryos.

MITOSIS

New cells (daughter cells) are formed by cell division. Cell division is initiated by the nucleus, and nuclear division is the first visible sign of the process of cell division. Mitosis is the commonest form of nuclear division, and results in two genetically identical daughter nuclei being formed. Mitosis occurs in the cell division seen in growth, repair, the replacement of cells which have a limited life span like our red blood cells and skin cells, and in asexual reproduction in many plants and lower animals. In the case of single celled organisms this type of asexual reproduction is called binary fission. The development of a multicellular organism from a fertilised egg by mitosis, means that all the cells of the body are genetically identical to the fertilised egg. The development of an organism from the fertilised egg is the story of the controlled expression of this genetic information, with only a small fraction of the genes being expressed in cells as they differentiate to form tissues specialised for specific (and limited) functions. In mature cells most of the DNA is inactive all of the time.

Asexual reproduction does not involve the fertilisation of gametes, and as a result of mitosis produces offspring which are genetically identical to each other (clones). Many multicellular organisms, especially plants can reproduce their entire bodies in this way, either from specialised structures, or single celled spores.

The production of genetically identical cells in the growth and development of multicellular organisms allows certain cells to retain the ability to develop into any other type if needed as a result of some damage. For example, stem cells in mammals can divide to form any of the different types of blood cell. In the asexual reproduction of organisms mitosis allows for the rapid exploitation of optimal conditions, where any variation would be disadvantageous.

The main stages of mitosis

Chromosomes only form and become visible (when stained and viewed under the light microscope) when the nucleus of a cell is just about to divide. This is because only at this time is the DNA/histone mixture wound up (condensed) as tightly as it can be. By this stage the DNA has also copied itself (replicated) so each chromosome appears as a double structure, joined at one point (the centromere). Whilst they remain joined in this way the two daughter chromosomes are referred to as chromatids.

Nuclear division (mitosis) occurs in four main stages, namely prophase, metaphase, anaphase and telophase.

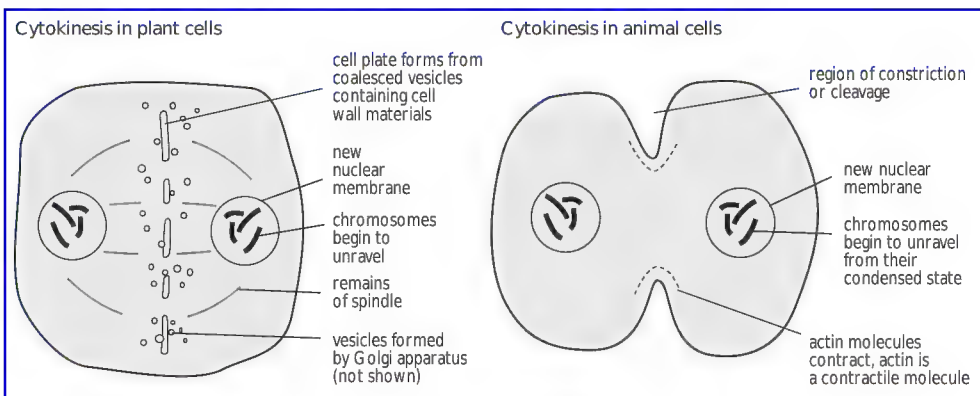
Prophase is the first phase of mitosis, and as it begins, the DNA has already replicated, all protein synthesis has stopped, and each chromosome is condensed into the characteristic double chromatid form. At the same time the cell rounds up, the nuclear membrane breaks down into small fragments, and a **spindle** of microtubules forms between two microtubule organising centres (MTOCs) at opposite poles of the cell.

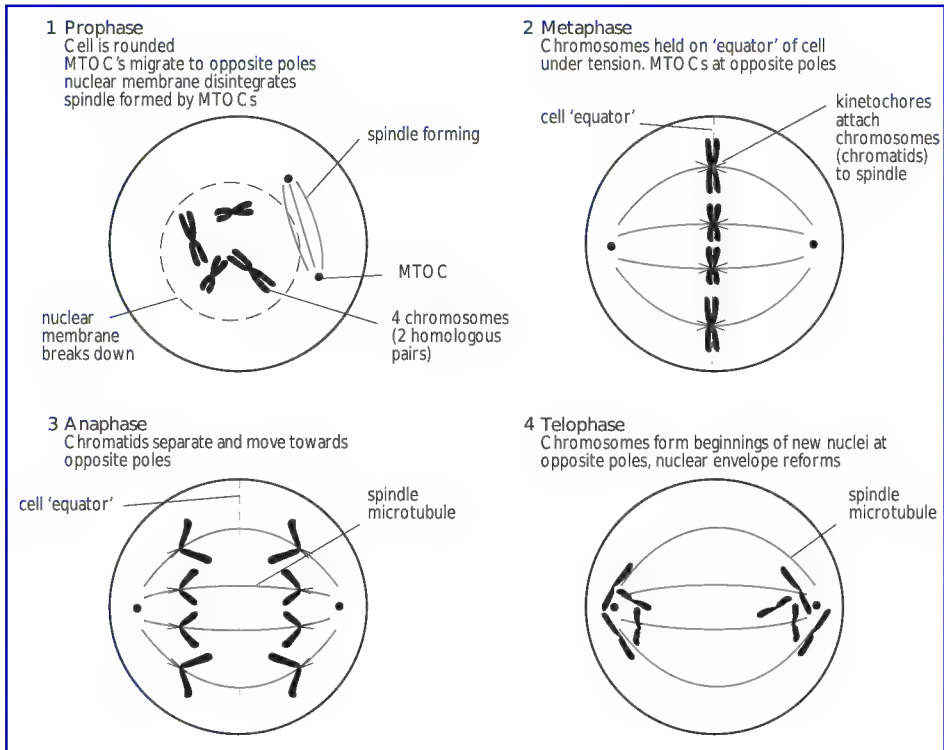
Metaphase is defined as when the chromosomes become attached to the spindle by structures called centromeres (kinetochores). Kinetochores consist of microtubules and 'motor' proteins which utilise ATP energy to pull on the spindle. The effect of both sets of kinetochores pulling in opposite directions is that the chromosomes line up along the middle (equator) of the spindle.

Anaphase occurs when quite suddenly, and all at once, the kinetochores and chromatids of each double chromosome separate, with each chromatid (now daughter chromosome) being pulled under tension in opposite directions towards the poles of the cell by the kinetochores, with their arms trailing in the characteristic 'V' shape.

Telophase is the stage when the two new, identical sets of chromosomes reach the opposite poles and a new nuclear envelope forms to surround each set.

Nuclear division by mitosis is followed by division of the cytoplasm into two equal parts. This process is called **cytokinesis** and it occurs differently in animals and plants.





In animal cells the spindle disintegrates during telophase and the microtubule components reassemble into a new cytoskeleton. The equator of the cell is constricted by a ring of contractile proteins (actin) in the process of cleavage, to create two roughly equal cells.

In plant cells the spindle lingers a little longer and seems to act as a guide to bring Golgi apparatus and associated secretory vesicles to the equator. The vesicles deposit their contents, which are the building constituents of a new cell wall, on the equator to form a plate of deposited material (the cell plate). Some of the vesicles remain intact and make connecting channels through the new cell wall (plasmodesmata).

The cell cycle

Interphase refers to the greater part of the life of a cell when there is no apparent activity. The period of mitosis and cytokinesis occupy only a fraction of the entire life of a cell. Together, interphase, mitosis and cytokinesis make up the **cell cycle**. The term interphase should not be interpreted to mean that the cell is resting. Interphase can itself be divided into three distinct periods called G₁, S and G₂. ('G' refers to the 'gaps' between DNA replication and mitosis.)

G₁ is by far the longest period of the cell cycle. During G₁, the cell machinery carries out protein synthesis, and growth occurs. All the cells of the body have identical sets of genes in their nuclei but the cells develop in different ways to perform different functions. This development process is called cell differentiation in which different genes are switched on and off, so that only one small part of the genetic code is active in each cell.

After a period of growth and differentiation, the length of which depends upon a variety of internal and external factors, protein synthesis is suspended, and the DNA set replicates itself. This is the **S** phase. DNA replication is followed by another 'gap' period, **G₂**, before mitosis starts, in which the cytoskeleton of the cell breaks down and the microtubule components begin to reassemble into spindle parts. Without a cytoskeleton, the cell assumes a more spherical shape (see prophase).

The timing of events within the cell cycle is extremely variable. In ideal conditions, where food and oxygen are in plentiful supply and temperature and pH values are optimal, bacterial cells can complete the cycle to produce a new generation every 20 minutes. Other cells, for example muscle cells, never complete the cycle, a condition called terminal differentiation.

It has become clear that the onset of the S (DNA replication) phase and the M (mitotic) phase are determined by genes which are very similar in all organisms. These genes direct the synthesis of enzymes which in turn activate the enzymes initiating S and M phases.

The rate of cell division can be controlled by naturally occurring plant substances which affect the process of nuclear spindle formation. These include vincristine and vinblastine (from the periwinkle plant *Catharanthus rosea*). Both of these substances are used to control cancers such as leukemia and lymphoma. They work, at a molecular level, by binding on to the spindle microtubules, preventing spindle assembly.

The plant substance, taxol, from the Pacific Yew (*Taxus brevifolia*) has almost the reverse effect on spindle formation, causing an over production of microtubules which effectively stops cell division. Taxol has been used to treat ovarian cancers. As more is known about the factors controlling the cell cycle, new possibilities will open up for drug therapy which can be more specifically targeted. Work in this field will be aided by the research which has discovered the sequence of bases in the DNA of the human chromosomes (the Human Genome Project) as it reveals more and more about the predisposition of individuals with particular gene combinations to cancer.

◆ CHECKPOINT SUMMARY

- ◆ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei.
- ◆ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur).
- ◆ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ◆ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical.
- ◆ Similarly, offspring of asexual reproduction lack genetic variation.
- ◆ The production of genetically identical cells with the full set of genetic information allows the replacement of any cells in repair processes. In the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.
- ◆ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere.
- ◆ The nuclear spindle apparatus(NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ◆ The centromeres align on the equator of the NSA (metaphase).
- ◆ The centromeres divide and the chromatids are moved to the opposite poles (anaphase).
- ◆ Two new daughter nuclei are formed (telophase).

Applications of cloning

Clones are genetically identical organisms. Cloning may occur naturally, for example, identical twins in humans, binary fission in microorganisms and vegetative reproduction in plants, but it can also be achieved artificially. The advantages to agriculture of cloning techniques are obvious; to be able to select the very best crop plant or farm animal and multiply it indefinitely has been the dream and ambition of farmers throughout the ages. Plant growers, since the earliest times, have used the natural properties of plants to multiply by vegetative (asexual) means; indeed some common crop plants, for example the banana, can only be grown by asexual **vegetative reproduction**. The cloning of animals is a relatively recent phenomenon, made possible by new techniques for manipulating embryonic cells, and whilst it opens up some exciting new biotechnological horizons, for many it also raises new ethical and legal questions.

Producing crops by vegetative propagation

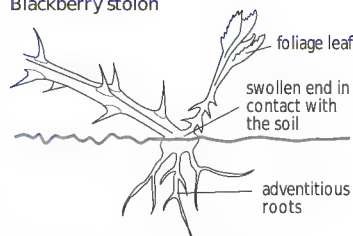
Most plants can reproduce asexually, as anyone who has planted a sprouting potato, or taken a geranium cutting will know. This kind of reproduction, which does not involve sex cells (gametes) and the production of seeds, results in genetically identical copies, or clones, of the parent plant and is called vegetative reproduction. Over the centuries, crop growers have exploited the natural asexual reproductive properties of plants to multiply selected varieties. The multiplication of plants by means other than the planting of seeds is termed **vegetative propagation**. Many important food crops such as grapes, olives, figs, potatoes, yams, sugar cane and bananas have been developed almost entirely by vegetative propagation.

There are obvious advantages in selecting a single superior individual from a plant population and reproducing it asexually. Since members of a clone are genetically uniform under optimum conditions, they grow to the same height, flower at the same time, and produce fruit of a similar size, features which make them highly suitable for mechanised farming. Vegetative propagation may be used to multiply infertile plants which have arisen from chance mutations but have commercial value, e.g. seedless fruits.

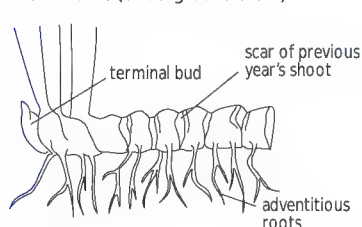
There are, of course, inherent risks in growing large numbers of plants with identical genotypes. The uniformity which makes them so attractive to farmers applies also to their susceptibility to pests, diseases and other environmental hazards. An entire crop may be wiped out by a single type of pathogen. Another drawback is the cost. By comparison with seed planting, it is more expensive per plant because it is more labour intensive, and it requires a large capital investment for research and technical facilities. Some plants, notably apple, pear, peach and cherry trees, have proved impossible to multiply by cuttings, but may be propagated vegetatively by the technique of grafting shoots onto root stocks of other types.

Clones may be propagated on for many generations. The Cabernet Sauvignon grape, for example, has been cloned for at least 2000 years, but repeated vegetative propagation can bring about serious disease problems. Without the natural break provided by seed formation and the opportunity for new virus-free seedlings each generation, cloned plants may retain their disease organisms from

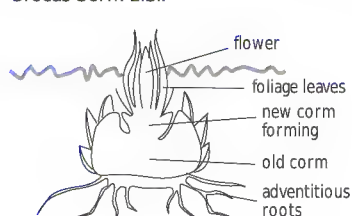
Blackberry stolon



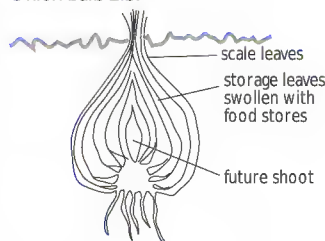
Iris rhizome (underground stem)



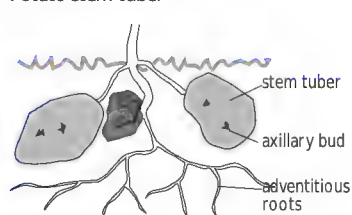
Crocus Corm L.S.



Onion bulb L.S.



Potato stem tuber



year to year, and these pathogens may evolve into more virulent strains. For example the virus diseases of potato accumulate so that there is a steady decline in productivity over the years in crops repeatedly grown from the preceding year's crop. Therefore 'seed' potatoes must be brought in from virus free reservoirs which are maintained in regions of high wind and rainfall (Scotland and Ireland) where the spread of virus diseases by aphids (greenfly) is prevented.

It is now possible to clone plants from cell cultures through the techniques of **micropropagation**. In some cases, whole new plants can be propagated from single cells usually isolated from the growing tips (apical meristems). New varieties may be cultured from single genetically engineered isolated cells, and multiplied rapidly. Plants which are transformed in this way to contain DNA from other organisms are called **transgenic plants**.

The cloning of animals

Identical animal clones can be produced by simulating the natural process by which identical twins are formed, in other words, by splitting apart the cells of developing embryos at a very early stage after fertilisation. Cows or sheep selected for a particular, desired characteristic may be inseminated artificially with semen from a selected bull and the embryonic ball of cells (**blastula**) is collected from the cow before it naturally implants into the wall of the uterus. Under a microscope the blastula can be divided into two or more 'twins' which are cultured on for a few days then implanted into a substitute (surrogate) mother treated with hormones so that her oestrous cycle coincides exactly with the real mother. Alternatively, the blastula can be frozen in liquid nitrogen and stored for later transfer. This technique is called **embryo transfer**.

The use of fertility drugs based on the female hormone FSH (follicle stimulating hormone) can increase the productivity of this process by stimulating multiple ovulation, hence multiple fertilisation and multiple blastulas. Fertilisation may be better controlled by harvesting the unfertilised eggs and mixing them with selected semen in the laboratory, a process, also performed with humans, called *in vitro* fertilisation. (Babies born by this method are commonly referred to as 'test tube babies') It is possible to sex the embryos of cattle and sheep before implantation so that only the desired ratio of females to males is produced.

A rather more sophisticated extension of these cloning techniques with farm animals is the **nuclear transfer** procedure. Unfertilised eggs are harvested from a donor animal and their nuclei are removed by suction with a micropipette. The 'enucleated' egg cells may then be fused with single cells taken from a selected blastula. Fusion is normally achieved by applying a small electric current to the culture medium. The egg, now equipped with a super selected genotype, is grown in culture for a few days, then implanted into a surrogate mother, or frozen. The advantage of this method is that the same selected blastula may yield 30 or 40 cells for nuclear transfer, each of which can be implanted into surrogate mothers and grow into an adult. Furthermore, the original donor may be treated with fertility hormones to repeat the process every three months.

The technique of nuclear fusion described above has been used to insert the genotype of an adult body cell into an unfertilised egg,

then brought to birth in a surrogate mother. Dolly, the famous sheep, was cloned in this way from cells taken from the mammary glands of its genetic mother.

Unlike plant cells, animal cells can not be induced to grow into whole new organisms once they have begun the process of differentiation. The only time this is possible therefore is before the onset of differentiation. The possibilities for creating transgenic animals are therefore very much more limited in animals than in plants. Now that the procedures for embryo transfer and nuclear transfer are improving, the possibilities for genetic modification and the creation of transgenic animals are rapidly expanding. Pigs, genetically modified to reduce the rejection response from a human organ recipient, may soon be cloned successfully to provide kidneys and other organs for transplantation. Transplantation of organs between animals of different species is called **xenotransplantation**. This is another field of research which raises ethical issues of public concern over which much debate will surely follow.

◆ CHECKPOINT SUMMARY

- ◆ Clones are genetically identical organisms.
- ◆ Cloning may occur naturally, for example, identical twins in humans, binary fission in microorganisms and vegetative reproduction in plants, but it can also be achieved artificially.
- ◆ The production of crops by vegetative propagation e.g. in which cuttings are taken from a desirable original is a form of cloning, some common crop plants, for example the banana, can only be grown by cloning.
- ◆ Since members of a clone are genetically uniform under optimum conditions, they grow to the same height, flower at the same time, and produce fruit of a similar size, features which make them highly suitable for mechanised farming.
- ◆ The uniformity which makes them so attractive to farmers applies also to their susceptibility to pests, diseases and other environmental hazards. An entire crop may be wiped out by a single type of pathogen.
- ◆ It is now possible to clone plants from cell cultures through the techniques of micropropagation. In some cases, whole new plants can be propagated from single cells.
- ◆ This is useful in producing virus free stock from apical meristem cells which viruses do not penetrate.
- ◆ The cloning of animals is possible by new techniques for manipulating embryonic cells, e.g. by splitting apart the cells of developing embryos at a very early stage after fertilisation.

2.3

Sexual Reproduction

Gametes and fertilisation

- ▼ Sexual reproduction involving gamete formation and fertilisation, and the transmission of DNA from one generation to the next by gametes.
- ▼ Differences between male and female gametes in terms of size, number produced and mobility.

Meiosis

- ▼ The separation of homologous chromosomes to produce gametes containing one chromosome from each homologous pair.
- ▼ The reduction of the number of chromosomes from the diploid number ($2n$) to the haploid number (n).

Importance of meiosis

- ▼ The maintenance of a constant chromosome number from generation to generation.
- ▼ The interpretation of life cycles of organisms in terms of mitosis, meiosis, fertilisation and chromosome number.

Introduction

Reproduction, the production of a new generation of organisms, is a characteristic feature of all living organisms. It involves the transmission of genetic information from one generation to the next and is clearly essential to the survival of a species. Without efficient reproductive strategies, organisms would soon become extinct.

Reproduction may be divided to two main types: asexual and sexual.

Asexual reproduction involves one parent only, and does not involve the formation of gametes. Offspring are genetically identical to the parent.

Sexual reproduction involves the production of haploid gametes, which are 'sex cells' containing half the DNA (one set of chromosomes) of the parent cells. Fusion of two gametes, at fertilisation, creates a diploid zygote (with two sets of chromosomes) which develops into a new individual. The gametes may come from one hermaphrodite parent or two parents, one male and one female. The offspring produced are never genetically identical to the parent, creating genetic variation within a species.

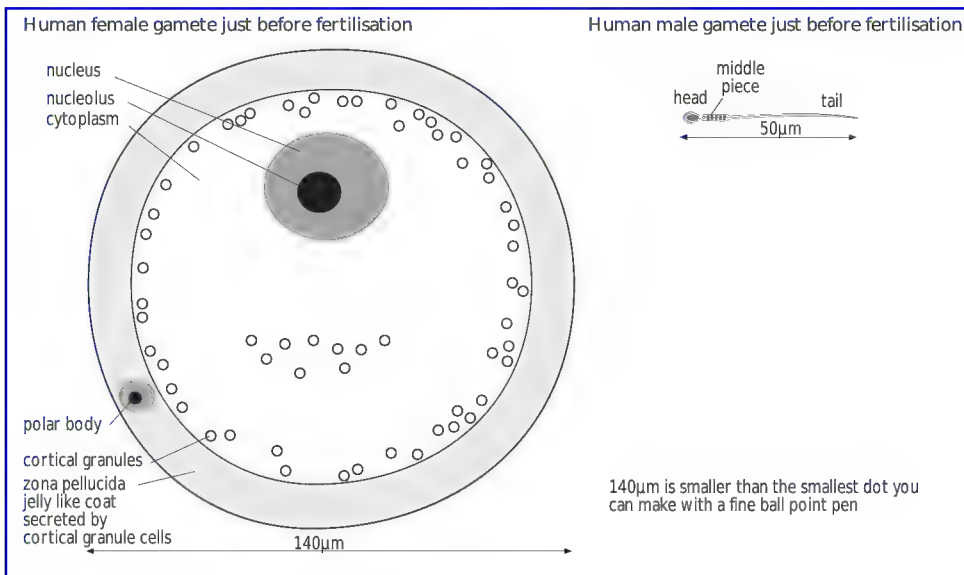
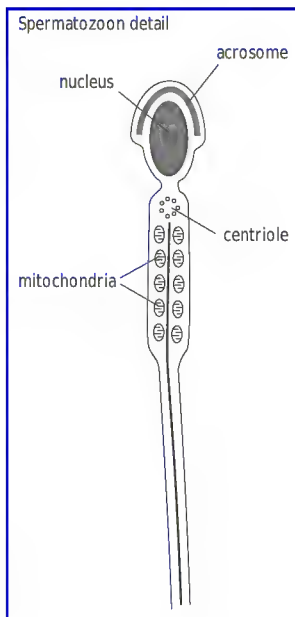
GAMETES AND FERTILISATION

In most sexually reproducing animal and plant species there are separate male and female gametes with distinctive characteristics. These may be produced by separate male and female organisms (as in mammals and some flowering plants e.g. holly) or by a single individual which contains both male and female organs (a hermaphrodite) as in some annelid worms and in most flowering plants. In the former case a gamete from a male parent must fuse with a gamete from a female parent to create a new individual.

Hermaphrodite organisms may be self-fertilising, requiring only one parent for sexual reproduction, or may have developed mechanisms to ensure that their gametes will only fuse with gametes from another individual. In most species gametes are only produced at certain times of the year (breeding seasons) designed to ensure the best conditions for the development of the young.

Male and female gametes typically differ from each other in a number of ways. These differences are particularly well illustrated in the mammals where internal fertilisation has led to exaggeration of some of the characteristics.

Size: Female gametes are usually much larger than male gametes. In the case of the human gametes the ovum is more than 50 times larger than the sperm cell. The main reason for this is that the female gamete typically contains a supply of stored food for use by the zygote in the first few days of development, but the egg cytoplasm is also vital for the expression of the genetic code in the production of a new individual.



Numbers produced: In general male gametes are produced in larger numbers than female gametes, particularly in mammals. In humans about 300 million sperm are present in semen from a single ejaculation. In contrast only about 300-400 ova will develop to maturity in a female during her whole reproductive life time. This partly relates to the fact that female gametes are larger and more expensive to produce in energy terms. In addition, many male gametes are needed since the chance of any individual male gamete finding and fusing with a female gamete is very low.

Mobility: Male gametes tend to be motile (capable of independent movement) whereas female gametes are not. This allows the male gametes to move towards the female gametes either within the body of an animal (e.g. human) or in the external environment (e.g. fish). To allow this the male gamete has a specialised structure, usually consisting of a tail for swimming, and many mitochondria for the supply of energy.

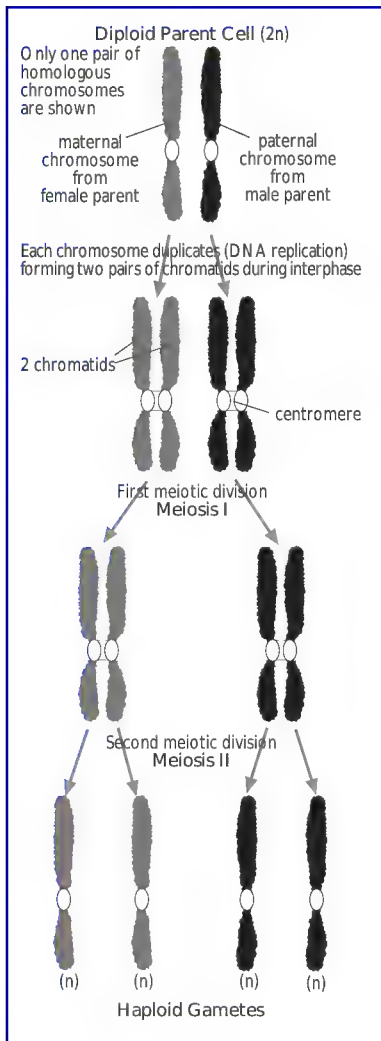
MEIOSIS

Meiosis is a special type of nuclear division called a reduction division in which the number of chromosomes is halved. In biological terminology the diploid chromosome number ($2n$) is reduced to the haploid chromosome number (n). This type of division only occurs in the production of gametes for sexual reproduction in animals, and spore production in plants. In the human it therefore occurs only in certain cells of the testis where sperm are produced and in certain cells of the ovary where ova are produced. As a result of meiosis human sperm and ova contain 23 chromosomes compared to the 46 chromosomes (23 pairs) found in all other cells of the human body. This means that when two gametes fuse the diploid number is restored (in humans $23 + 23 = 46$).

As with mitosis, meiosis involves several stages which begin with interphase a period during which the DNA of the cell and its organelles are replicated. There are, however, several significant differences between the two types of division. In mitosis a cell divides to produce two identical daughter cells with the same number of chromosomes (diploid $\rightarrow$ diploid). In meiosis a cell goes through two cycles of nuclear division and divides to form four non-identical daughter cells, each with half the chromosome number (diploid $\rightarrow$ haploid).

In every cell there are two copies of each chromosome which are the same size and shape as each other (termed homologous) and carry the same genes. One of these chromosomes originally came from the female parent and one from the male parent of the individual. In humans there are 23 pairs of chromosomes (two copies of chromosome 1, two copies of chromosome 2, two copies of chromosome 3 etc.). Meiosis must ensure that members of these pairs of homologous chromosomes separate so that each gamete receives only one copy of each chromosome rather than two (i.e. one copy of chromosome 1, one copy of chromosome 2 etc.).

To achieve this the chromosomes pair up in their homologous pairs at the beginning of meiosis. They then line up together on the equator of the spindle (see section 11.2), and then one chromosome from each homologous pair is pulled into one new cell (gamete) whilst the other one of the pair ends up in the other new cell.



◆ CHECKPOINT SUMMARY

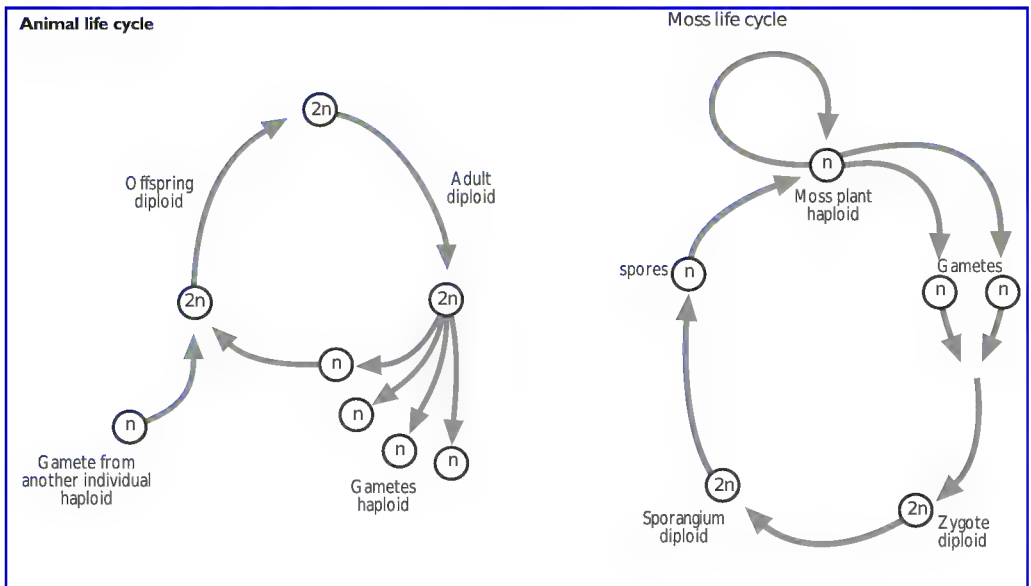
- ◆ In sexual reproduction DNA from one generation is passed from one generation to the next by sex cells (gametes) which fuse their nuclei in fertilisation.
- ◆ Male gametes are smaller, much more numerous, and motile, finding their way to the fewer, larger; immobile female gamete(s) in a variety of ways.
- ◆ The male gamete contributes only its nucleus in the process of fertilisation, the cytoplasm of the female gamete is vital in the development of the fertilised egg into a multicellular organism.
- ◆ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid).
- ◆ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid).
- ◆ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes).
- ◆ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid.
- ◆ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.
- ◆ Life cycles of sexually reproducing organisms therefore involve mitosis, meiosis, fertilisation and changes in chromosome number.
- ◆ Animals and flowering plants are diploid with meiosis forming haploid gametes, but mosses and their relatives are haploid, gametes are produced without meiosis, and meiosis precedes spore formation. The haploid spores form new haploid moss plants.

The importance of meiosis

Meiosis is essential to the maintenance of constant chromosome number across generations. As described above, the full chromosome number is halved in the production of gametes and then restored when haploid gametes fuse to create a diploid zygote. If meiosis did not occur in the production of gametes, then every new zygote would have twice as many chromosomes as the parents. This would not only affect the appearance and characteristics of an organism, and disrupt normal nuclear and cell division, but would soon result in too much genetic material for individual cells to accommodate.

Note that mitosis is the main type of cell division throughout an individual's life time: it is only gamete production that requires meiosis.

Meiosis is also significant in generating genetic diversity since every gamete produced by an individual is different from every other. In humans the separation of homologous pairs in different ways creates a possible 2^{23} different genetic combinations of sperm or ova: no surprise then that two offspring from the same parents will always be different (unless they are identical twins originating from one pair of gametes).



2.4.

Applications of Gene Technology

Contents

Principles of genetic engineering

- ▼ The transfer of genes from one organism into another
- ▼ The use of restriction endonuclease enzymes to extract the relevant section of DNA
- ▼ The use of ligase enzyme to join this DNA into the DNA of another organism.

The polymerase chain reaction

- ▼ The process of artificial and repeated DNA replication in the laboratory by the polymerase chain reaction (PCR)
- ▼ The use of PCR, radioactive labelling and electrophoresis to determine the sequence of nucleotides in DNA.

Genetically engineered microorganisms

- ▼ Microorganisms as recipient cells during gene transfer
- ▼ Plasmids as vectors to incorporate selected genes into bacterial cells
- ▼ Use of microorganisms to clone a transferred gene.

Genetic markers

- ▼ Genetic markers in plasmids, such as genes which confer antibiotic resistance, and replica plating as techniques to detect the bacterial cells that contain genetically engineered plasmids.

Large scale culturing

- ▼ The culture of bacteria containing the transferred gene on a large scale in industrial fermenters
- ▼ Useful substances produced by using genetically engineered microorganisms, including antibiotics, hormones and enzymes.

Gene therapy and cystic fibrosis

- ▼ The cloning of healthy genes & their use in replacing defective genes
- ▼ The transmembrane regulator protein, CFTR mutation & its effects
- ▼ The symptoms of cystic fibrosis related to the malfunctioning of CFTR
- ▼ Techniques that might possibly be used to introduce healthy CFTR genes into lung epithelial cells including:
 - use of a harmless virus into which the CFTR gene has been inserted
 - wrapping the gene in lipid molecules that can pass through the membranes of lung cells.

Genetically modified animals

- ▼ Genetically engineered sheep to produce alpha-1-antitrypsin and the treatment of emphysema and cystic fibrosis.

Evaluation of genetic engineering

- ▼ The ethical, social and economic issues involved in the use of genetic engineering in medicine and in food production.

Introduction

In 1952 Hershey and Chase proved that DNA was the genetic material. The following year the double helix structure of DNA was elucidated, followed by the unravelling of the genetic code and the cloning of the first genes in the 1960s. From this point onwards the stage was set for direct human manipulation of DNA and the rise of DNA technology over the past four decades. Now, early in the twenty first century, the pharmaceutical and food industries are already reaping the benefits of genetically engineered organisms; trials are underway for gene therapy which might one day help to cure sufferers of severe genetic diseases; forensic science is benefiting from DNA fingerprinting; and evolutionary biologists are experimenting with the cloning of ancient DNA. In this rapidly developing branch of science there are many potential benefits to individuals and to society as a whole, and possibly many disadvantages.

PRINCIPLES OF GENETIC ENGINEERING

Genetic engineering is the manipulation of the DNA of an organism. It primarily involves removing genes from one organism (the donor) and inserting them into another organism (the recipient or host). The recipient organism is known as a **transgenic** organism. The donor organism may be of a different species, phylum, or even kingdom from that of the recipient organism. This is made possible by the fact that DNA has a universal code in all living organisms: genes are simply sequences of the four bases A, C, T and G whether they come from a human, a bacterium or a buttercup.

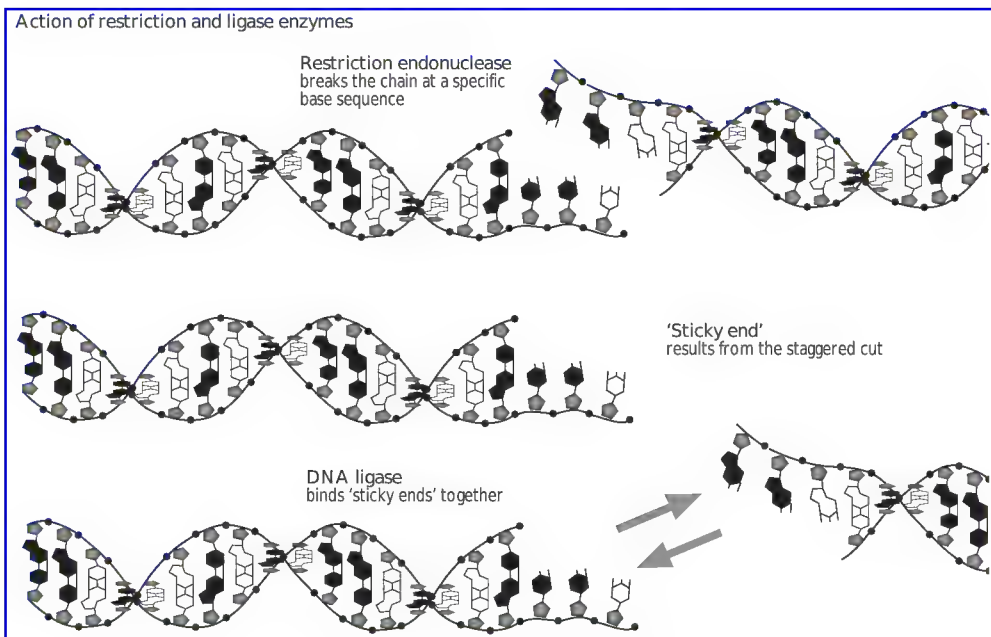
Genetic engineering achieved its first results in the 1970s using bacteria as the recipient organisms. Since that time a wide range of transgenic bacteria and fungi have been produced. More recently transgenic plants with features such as herbicide resistance, have begun to be grown on a large scale, and transgenic animals are being investigated for the production of medical products.

Genetic engineering involves several complex stages which differ depending on the organisms involved. All are highly specialised and technically difficult, but all centre around three basic stages:

- ▼ The desired gene must be found in the DNA of the donor organism or created from the mRNA of a known protein.
- ▼ The desired gene must be removed from the DNA of the donor using **restriction enzymes** (enzymes which cut DNA at specific points and allow removal of a small section: see below).
- ▼ The desired gene must be inserted into the DNA of the recipient organism. Having cut the DNA of the recipient organism using restriction enzymes, **ligase enzymes** are used to join the new gene permanently into its own DNA.

Restriction enzymes or restriction endonucleases are enzymes derived from bacteria which cut DNA at specific base sequences. Such enzymes will 'recognise' particular DNA sequences known as recognition sites, attach to these, and cut the DNA at specific restriction sites. There are several thousand types of restriction enzymes now in use whose names reflect the bacterial species from which they were extracted: thus EcoR1 is derived from E.Coli and HindI from *Haemophilis influenzae*. In nature these enzymes allow bacteria to cut out sections of unwanted DNA which have been inserted by viruses.

Each restriction enzyme is specific to one base sequence and will digest or 'cleave' the nucleic acid (DNA) whenever such a site is encountered but at no other point. Some cut straight through both strands of DNA creating 'blunt ends', whilst others produce a staggered cut or 'sticky end' which is useful in genetic engineering. In genetic engineering restriction enzymes must be chosen which cut the donor DNA either side of the desired gene. The same enzyme will then be used to cut the DNA of the recipient organism or vector to allow the gene to be inserted.



POLYMERASE CHAIN REACTION (PCR)

PCR is a technique used to amplify (make many copies of) a DNA molecule or part of a DNA molecule. Based on an understanding of the semi-conservative mode of DNA replication in nature, PCR has revolutionised genetic technology. It theoretically allows any number of copies of DNA to be produced from a single DNA molecule (the template). Often it is used to amplify a known section of DNA only. PCR is crucial in generating copies of DNA for use in such processes as: gene sequencing, genetic fingerprinting and genetic screening for diseases.

Amplifying a piece of DNA by PCR requires the 4 'ingredients' listed below.

- ▼ DNA. Theoretically a single DNA molecule is sufficient, but usually a larger amount of DNA is used.
- ▼ Primers: These are short single stranded pieces of DNA which bind to the parent DNA strands and initiate the synthesis of a complete new strand of DNA on the template of the parent strand. One primer is needed for each parent DNA strand.
- ▼ A supply of each of the 4 nucleotides containing the bases A, C, T and G. The bases on the free nucleotides pair with their complementary bases on the DNA parent strand (A with T and C with G) thus synthesising the new strand.
- ▼ The enzyme Taq polymerase. This enzyme works like human DNA polymerase, catalysing the attachment of the complementary bases to one another. Taq polymerase is, however, thermostable (temperature resistant) as it is derived from the bacterium, *Thermus aquaticus*, which inhabits hot springs. This is important as PCR requires high temperatures to work.

The reaction mixture made of these 4 ingredients is placed in an individual well on a plastic plate, or in individual tubes with a layer of oil on top to prevent evaporation. The reactions involve alternate heating and cooling of this mixture, and are performed by a PCR machine. The machine can be programmed for different numbers of cycle: the more cycles the more copies of the DNA are produced.

Sequence of events

For simplicity the stages of PCR are described here as if a single complete

DNA molecule is used as the starting point.

- a) PCR mixture is heated causing the DNA double helix to unwind exposing the bases.
- b) The mixture is cooled and primers bind to the ends of each DNA strand. This is known as annealing.
- c) Taq polymerase allows free nucleotides to pair with the exposed bases on both DNA strands. A pairs with T and C with G. Binding begins immediately after the primer sequence and continues until the whole complementary strand has been synthesised. Two new DNA double helices thus result. Each contains one of the original parent strands. One complete cycle has now been completed.
- d) The process is repeated starting with two copies of the DNA and ending with four. (Each DNA molecule unwinds, has a complementary strand synthesised and winds back up). As the

process is repeated over and over again the number of copies of the DNA increases geometrically: 2→4→8→16→32→64→128 etc. Several million copies may be made in the space of a few hours.

- e) The DNA produced is used in genetic fingerprinting, genetic screening or other genetic studies. Alternatively it may be frozen and stored for future analysis.

GENETICALLY ENGINEERED MICROORGANISMS

In biology the term microorganism is used to describe any single celled organism which can only be seen using a microscope. It therefore includes bacteria, unicellular fungi and some members of the protist kingdom. Many different types of bacteria and more recently some types of yeast (a single celled fungus) have been used in genetic engineering.

Bacteria are ideally suited to genetic engineering. One of the reasons for this is that they contain small circles of DNA known as plasmids which act as vectors by which new genes can be introduced into bacterial cells. The first stage of genetic engineering is to identify and isolate the desired gene from the donor organism. This is very difficult and involves a range of techniques beyond the scope of this text. Instead we will consider the process the stages from this point onwards, assuming that the desired gene is ready to be inserted:

Stage 1

Inserting genes into the plasmid vector

- The plasmids must be removed from bacterial cells. Bacteria cells are broken open using high temperatures or chemical means. The contents are centrifuged and plasmids purified.
- The plasmid DNA is cut open using a restriction enzyme. The same restriction enzyme is used as was used to cut the donor gene from donor DNA. In both cases 'sticky ends' are produced which helps the insertion of the gene.
- The donor genes are mixed with the plasmids.
- Some plasmids take up donor DNA. Attraction occurs between sticky ends but insertion of the gene is aided by ligase enzymes. The DNA of the plasmid is now called recombinant DNA.

Stage 2

Introducing vector DNA into the host cell (transformation)

- Plasmid vectors containing recombinant DNA added to a culture of bacteria (often *E.coli* bacteria are used at this stage although the plasmids may come from other species of bacteria.)
- Calcium ions and heat are used to make the cell membrane of the bacterium permeable to plasmids.
- Plasmids successfully enter some bacterial cells.

Stage 3

Cloning DNA (producing many copies of the recombinant DNA)

Bacteria from Stage 2 are then grown using suitable medium. Since bacteria reproduce at a very fast rate billions of cells can be created

◆ CHECKPOINT SUMMARY

- ◆ Genetic engineering is the manipulation of the DNA of an organism. It primarily involves removing genes from one organism (the donor) and inserting them into another organism (the recipient or host). The recipient organism is known as a transgenic organism.
- ◆ There are three basic stages:
- ◆ The desired gene must be located in the DNA of the donor organism or created from the mRNA of a known protein.
- ◆ The desired gene must be removed from the DNA of the donor using restriction enzymes which cut DNA at specific points and allow removal of a small section.
- ◆ The desired gene must be inserted into the DNA of the recipient organism. Having cut the DNA of the recipient organism using restriction enzymes, ligase enzymes are used to join the new gene permanently into its own DNA.
- ◆ The Polymerase Chain Reaction (PCR) is a technique used to amplify (make many copies of) a DNA molecule or part of a DNA molecule.
- ◆ PCR has revolutionised genetic technology. It theoretically allows any number of copies of DNA to be produced from a single DNA molecule (the template).
- ◆ PCR, radioactive labelling and electrophoresis in which fragments of different size are separated and identified allows the determination of the sequence of nucleotides in DNA.

in a short space of time. Every time the bacteria containing recombinant plasmids divide copies of the plasmid containing the desired gene are passed on. A single bacterium may have many copies of the plasmid, thus increasing the number of cloned genes present.

Stage 4

Selecting the transformed bacteria using GENETIC MARKERS

Stages 1 and 2 described above are far from perfect. In stage 1 many plasmids do not take up the donor DNA, and in stage 2 many bacteria do not take up plasmids. Of those bacteria that do take up plasmids, only those taking up plasmids containing the desired gene will be transformed. To check which bacteria from the clones grown in stage 3 have been transformed (and are hence useful for producing a product) a special technique involving marker genes for antibiotic resistance is often used.

Before being used as vectors, plasmids are produced which have two genes for antibiotic resistance. One gene will make the bacteria containing the plasmid resistant to the antibiotic ampicillin, and the other will confer resistance to the antibiotic tetracycline. When a plasmid takes up a gene from the donor organism, as described in stage 1, the gene is inserted in the middle of the tetracycline resistance gene sequence owing to the presence of restriction enzyme sites. This inactivates the tetracycline resistance gene. The ampicillin gene is unaffected.

Bacteria transformed by recombinant plasmids should therefore be resistant to ampicillin but will not grow on agar containing tetracycline. Bacteria which have taken up normal plasmids which do not contain recombinant DNA should be resistant to both antibiotics and will grow on agar containing both antibiotics.

Replica plating

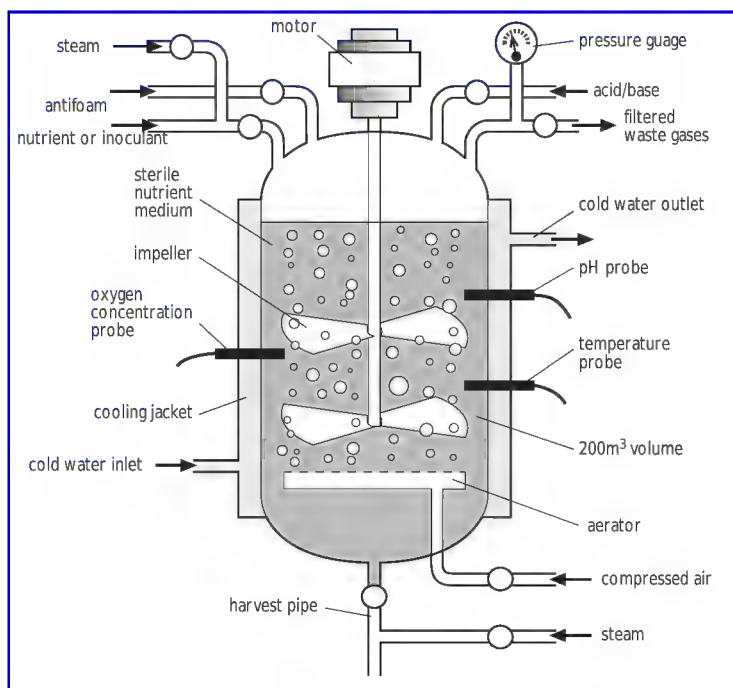
To determine which bacteria have been transformed the following steps are taken:

1. Bacteria from stage 3 above are placed onto an agar plate containing ampicillin.
2. When colonies of bacteria have developed, a sterile pad is placed on top of the colonies. This allows some bacteria from each colony to be picked up.
3. The pad containing the bacteria is pressed onto a second agar plate containing ampicillin and tetracycline. The use of the pad reproduces the same pattern of colonies on the new plate.
4. Colonies of transformed bacteria fail to grow and are destroyed by the antibiotic. Bacteria without recombinant plasmids grow.
5. Colonies of transformed bacteria on the original plate can now be identified. These are then cultured. The remaining colonies are discarded.
6. Once transformed bacteria have been selected they can then be cultured to produce the desired gene product. Techniques to maximise bacterial growth and hence production have developed alongside genetic engineering. A range of valuable enzymes, hormones and other proteins are now produced rapidly, cheaply and safely by microorganisms in fermenters.

LARGE SCALE CULTURING OF GENETICALLY MODIFIED MICROORGANISMS

Growth of bacteria in industrial fermenters (large specialised tanks) involves the same basic principles as the small-scale growth of microorganisms in a laboratory. Bacteria will only grow at their optimum rate if provided with the following basic conditions:

1. A suitable nutrient medium in which to grow. This should contain simple organic sources of carbon and nitrogen (such as simple sugars and amino acids) and a range of vitamins and minerals dissolved in water.
2. Suitable environmental conditions, including an optimum pH and temperature for enzyme activity. Oxygen may or may not be required. Toxic waste products which inhibit growth should be removed.
3. Complete absence of competition or predation from other microorganisms. To achieve this the fermenter is sterilised before use and all substances added are also sterile.



Note in particular the inlet pipes by which nutrients are added, the temperature and pH monitors, the measures to exclude other microorganisms, the outlet pipes by which the product is harvested and the paddle wheel to keep the contents well mixed. In initial stages of culturing genetically engineered microorganisms small fermenters with volumes of 2-200 litres are used. Once established, however, vessels up to 200,000 litres may be used. With fermenters of this size, several hundreds of tonnes of product can be generated per day.

There are two main types of fermentation: **batch** fermentation and **continuous** culture. Batch fermentation is where a single culture of microorganisms and a single batch of nutrient medium is used to produce one batch of product. The microorganisms are then discarded and the process begins again. In continuous culture a colony of microorganisms is maintained over a long period of time. Small quantities of nutrient medium are added continuously and the product is removed regularly.

A range of products are now produced in fermenters by genetically engineered microorganisms. Some of these are shown below.

Product/function	Microorganism involved	Uses
Insulin	Bacteria and yeast	To treat diabetes
Growth Hormone	Bacteria	Promote growth in children with deficiencies of pituitary gland
Bovine somatotrophin (BST)	Bacteria	Increase milk production in cattle
Penicillin (antibiotic)	Fungus	To treat bacterial infections
Hepatitis B antigens	Bacteria	Safe vaccine against hepatitis B.
Chymosin (an enzyme like rennin)	Bacteria	Cheese making (vegetarian cheese)

GENE THERAPY AND CYSTIC FIBROSIS

The replacement or manipulation of defective genes in the cells of individuals suffering from a variety of diseases represents a major goal in the application of genetic engineering technology to human health. Termed 'gene therapy' this branch of medicine is still at the research stage, although it is anticipated that it could revolutionise the treatment of genetic disease in the future by effectively curing the disease 'at source'. Initial work has been done with single gene disorders such as cystic fibrosis, but it has been suggested that gene therapy could extend to the treatment of some cancers and even HIV/AIDS.

There are two main approaches to gene therapy. The first, and the only one currently being investigated in humans is known as '**somatic gene therapy**'. This approach involves inserting 'normal' genes into the DNA of some of the body cells of an affected individual. Target cells might, for example, be those of the lung as described below. Whilst this could cure an individual of a genetic disease it would not affect the gametes produced and hence the inserted genes would not be passed on to future generations. In contrast, germ line gene therapy involves the introduction of genes into sex cells or fertilised egg cells. In this case the new gene would be found in all somatic cells and all germ cells and would thus change the genetic makeup of future individuals. The ethical implications of such germ line gene therapy are considerably greater than with the somatic form. For this reason research into germ line gene therapy in humans is currently banned.

◆ CHECKPOINT SUMMARY

- ◆ Genetically engineered microorganisms include many different types of bacteria and more recently some types of yeast
- ◆ Plasmids are often used as vectors to incorporate selected genes into bacterial cells.
- ◆ To check which bacteria from the clones have been transformed (and are hence useful for producing a product) a special technique involving marker genes for antibiotic resistance is often used.
- ◆ Bacteria transformed by recombinant plasmids being resistant to ampicillin but susceptible to tetracycline.
- ◆ Large scale culturing of genetically modified microorganisms involves techniques to maximise bacterial growth and hence production
- ◆ Once established vessels up to 200 000 litres may be used. With fermenters of this size, several hundred tonnes of product can be generated per day.
- ◆ There are two main types of fermentation: batch fermentation and continuous culture. Batch fermentation is where a single culture of microorganisms and a single batch of nutrient medium is used to produce one batch of product.

The procedure of gene therapy involves three main stages, once the cause of a genetic disease has been established and the gene or genes responsible located.

- ▼ Firstly the normal gene must be isolated, removed from a human cell and cloned using bacterial cloning methods already described.
- ▼ Secondly the normal gene(s) must be inserted into a suitable vector. Possible choices of vectors are: viruses, liposomes and naked DNA.
- ▼ Thirdly the genetic material must be transferred into the patient. In some cases, as with defects of the blood cells this may be done outside the body using cells extracted from the patient which can then be modified and returned. In other cases the DNA must be delivered into the body of the patient via aerosols or other methods.

Cystic fibrosis

Cystic fibrosis is one of the most common non-sex chromosome (autosomal) recessive genetic diseases and affects about 1/2000 babies born in the UK. About 1:23 of the population are carriers of the cystic fibrosis allele which is found on chromosome 7. The disease which primarily affects the lungs, pancreas and liver is characterised by the production of thick, sticky mucus by epithelial cells in these regions. As well as causing severe breathing difficulties the mucus also leads to numerous bacterial infections. In spite of antibiotics and other drug treatments and regular physiotherapy to help move the mucus out of the lungs, CF usually causes death by early adulthood. CF is caused by a faulty protein known as CFTR (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells. A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced. This change in the primary structure of the protein alters its secondary and tertiary structures, preventing it from responding to signals to open chloride channels in cells. The overall effect of this is a build up of ions inside the cells which prevents water leaving by osmosis as it would normally do. Thus mucus released by cells is thicker than in normal individuals and sticks to cells causing inflammation and tissue damage, encouraging infections and preventing normal functioning of the organs concerned.

Owing to the lack of effective treatments available to CF sufferers there has been much interest in gene therapy for this disease. The aim in this case is to replace the faulty CFTR gene in lung cells with the normal gene for CFTR production along with its control sequence, thus allowing production of the normal protein in cells of the lungs. Two delivery techniques have so far been tried to deliver cloned copies of the CFTR gene to human cells.

- ▼ delivery via an adenovirus vector. This is a DNA virus of the family which causes colds. The virus has its pathogenic (disease causing) genes removed and the DNA for CFTR inserted. When the virus invades cells the DNA for CFTR is released.

Advantages of this system are that the vector will readily infect non-dividing cells and has a natural attraction to cells of the lung region. Disadvantages are that the virus may be attacked by the immune system, and that the DNA is unstable.

▼ delivery via liposomes. These particles are created in the laboratory by the fusion of the CFTR DNA (negatively charged) with positively charged lipid particles. They are taken up by the cell surface membrane of lung cells and the CFTR DNA can become functional inside the cell.

The advantage of this method is that it doesn't cause an immune response, but the disadvantage is that it is a very inefficient means of delivering DNA since most of it gets destroyed by lysosomes.

Both delivery techniques were first tried out in vitro (in the laboratory) using extracted lung cells, and then in experimental animals (where the CFTR gene had been removed). More recently trials in small numbers of humans have been performed. In these the adenovirus vector was used to deliver the CFTR gene, via an aerosol spray, to cells in the nasal passages and lower respiratory tract. Liposome vectors have so far only been used to deliver the gene to the nasal passages, also via an aerosol spray.

Human trials have provided evidence of gene transfer taking place between vector and human cells in up to 50% of cases. In addition, there has been more limited evidence of production of functional CFTR protein correcting the defect. In all cases where functional improvement occurred this was restricted to a period of 7-10 days which can be explained by the fact that the cells targeted by gene therapy do not divide and consequently will die and be lost. Thus whilst some progress has been made into gene therapy for CF, cure of this disease is still a long way off.

GENETICALLY MODIFIED ANIMALS

Several human proteins of great medical value are now produced by genetically engineered micro-organisms. In some cases, however, microorganisms are not well adapted to this task often because they lack specific enzymes which are required to modify the proteins produced and allow them to become biologically active. For this reason, scientists have developed techniques of genetically engineering mammals (farm animals) to produce human proteins. Such animals are sometimes termed 'animal bioreactors'.

In contrast to work with microorganisms, insertion of human genes into mammalian egg cells is very difficult and costly, whilst the care of farm animals is expensive and their reproductive potential limited. For these reasons such techniques must allow continued production and easy extraction of the protein without killing or harming the animal. The ideal solution is to have the protein secreted in milk. To achieve this the human gene is attached to a gene control sequence from the recipient mammal which ensures that the gene will only be 'switched on' in the tissues of the mammary gland.

Production of alpha-1 antitrypsin

Alpha-1 antitrypsin is a protein which is produced in the human liver and transported in the plasma. Its main function is to inhibit the enzyme elastase which is produced by white blood cells. If this enzyme is not inhibited (owing to a hereditary deficiency of alpha-1 antitrypsin) a form of early onset emphysema can develop whereby alveoli are destroyed and lung function considerably reduced. These patients make up a small percentage of emphysema patients and may or may not be smokers.

In recent years sheep have been genetically engineered to produce the alpha-1 antitrypsin enzyme using the procedure outlined below. Alpha-1 antitrypsin enzyme now makes up half of the protein content of the milk of these transgenic sheep. The enzyme can be easily extracted. Whilst clinical trials of this enzyme in humans is still awaited (May 2000) it has the potential to improve the treatment of patients with emphysema and cystic fibrosis. Currently these patients rely on alpha-1 antitrypsin enzyme extracted from human blood.

Stages in the production of alpha-1 antitrypsin by transgenic sheep

- ▼ Locate gene for alpha-1 antitrypsin and its control sequence in DNA of human liver cells
- ▼ Cut gene from DNA using restriction endonuclease
- ▼ Copy gene using PCR techniques
- ▼ Separate gene and control sequence using restriction enzymes
- ▼ Locate B-lactoglobulin gene and its control sequence in DNA from mammary cells of sheep
- ▼ Cut gene from DNA using restriction endonuclease and clone gene using PCR techniques
- ▼ Separate gene and control sequence using restriction enzymes
- ▼ Attach control sequence DNA from B-lactoglobulin to alpha-1 antitrypsin gene
- ▼ Use microinjection to deliver copies of hybrid transgene to fertilised sheep ovum
- ▼ Test for production of alpha-1 antitrypsin in milk of adult sheep using protein electrophoresis.

◆ CHECKPOINT SUMMARY

- ◆ Somatic gene therapy' involves inserting 'normal' genes into the DNA of some of the body cells of an affected individual. Target cells are the lungs in cystic fibrosis.
- ◆ Firstly the normal gene must be isolated, removed from a human cell and cloned.
- ◆ Secondly the normal gene(s) in the form of cDNA must be inserted into a suitable vector. Possible choices of vectors are: retroviruses, adenoviruses, liposomes and naked DNA.
- ◆ Thirdly the genetic material must be transferred into the patient.
- ◆ Cystic fibrosis is caused by a faulty protein known as CFTR (cystic fibrosis transmembrane regulator) which regulates transmembrane chloride ion channels in epithelial cells. A deletion of 3 bases in the DNA code of the CFTR gene means that one amino acid (phenylalanine) is missing from the CFTR protein which is produced.
- ◆ The overall effect of this is a build up of ions inside the cells which prevents water leaving by osmosis as it would normally do. Thus mucus released by cells is thicker than in normal individuals and sticks to cells causing inflammation and tissue damage, encouraging infections and preventing normal functioning of the organs concerned.
- ◆ Techniques to introduce healthy CFTR genes into lung epithelial cells include, delivery via a DNA virus of the family which causes colds; and wrapping the CFTR gene in lipid molecules. They are taken up by the cell surface membrane of lung cells and the cDNA can become functional inside the cell.
- ◆ Animals, e.g. sheep have been genetically modified to produce the alpha-1 antitrypsin enzyme used in the treatment of patients with emphysema and cystic fibrosis.

EVALUATION OF GENETIC ENGINEERING

Ethical, social and economic issues

In considering genetic engineering in its social and economic context it is worth reflecting on the fact that some kind of genetic modification of plants and animals has been in operation for thousands of years. Whilst early farmers did not know about genes or, of course, genetic engineering, their techniques of selective breeding and hybridisation dramatically altered gene frequencies and gene combinations in domestic organisms. The resultant changes in appearance and characteristics helped to create our familiar high yielding cereals, large, sweet fruits, and cows with high milk yields. In view of this it could be said that genetic engineering is at the modern end of a continuum of genetic change brought about by humans.

Yet for a variety of reasons the current methods of genetically modifying (GM) organisms, particularly plants and animals, is opposed by many people. Some likely factors behind this are the speed with which genetic engineering can operate, the potential for transferring genes between very different organisms and the greater public awareness of the process owing to media attention.

Food production One of the main arguments against GM foods is the concern over possible health risks. It is argued that plants containing genes from other organisms including in some cases bacteria and viruses may be unsafe, although there is no strong scientific evidence to suggest that there is any risk from foods currently in use.

GM crop plants are at the centre of a debate about the possibility of cross-pollination of wild plants, so spreading genes into these organisms. If, for example, genes for herbicide resistance were transferred to weeds then weeds might spread rapidly, compete with crops and upset natural food chains. On the positive side, however, it is argued that GM crops may actually benefit wildlife. If crops need fewer chemical herbicides, pesticides and fertilisers it could reduce pollution and the unnecessary killing of harmless organisms. Although, one of the first widely planted GM crops is a Maize which is resistant to a certain brand of herbicide.

The possible hazard of genetically engineered micro-organisms, including viruses, escaping from laboratories also raises safety issues. One particular worry is that bacteria used for a range of genetic engineering purposes often carry genes for antibiotic resistance which are used as markers. Since bacteria can exchange genetic material, they could transmit these genes to disease causing organisms which may then be resistant to treatment, adding to the already growing problem of natural resistance.

A further issue raised by the current debates on genetically engineered foods is the idea of consumer choice. It is argued that individuals should be able to choose whether or not to buy food containing GM ingredients, yet labelling of products does not make this possible. In other ways, though, genetic engineering can increase consumer choice: products such as 'Quorn' (a high protein meat substitute produced by a fungus) and vegetarian cheese (produced using a form of rennin from genetically engineered bacteria) were not available before genetic engineering. In addition GM foods may be cheaper, and for many people cost is the most important factor to consider.

Genetic engineering has also had an impact on animal products mainly via the use of genetically engineered molecules such as

growth hormones. Several years ago public outcry led to the banning (in Europe) of the hormone BST which was being used to increase milk yield in cattle. There was concern in this case over the well being of the cattle as well as over possible risks to human health.

One of the major economic arguments for genetically modified foods, particularly cereal crops, is the need for increased food supplies to feed an expanding world population. On one level this may be true since genetically engineered crops often have higher yields, more nutritious tissue, and cheaper production and processing costs than conventional crops. Yet whilst this may benefit customers in some countries it would not necessarily mean more food for individuals in developing countries since it is often the distribution of food in such countries which is the main problem. Furthermore, economic gain from such crops may also not benefit individuals in developing countries since their production may be run by multinational companies who hold patents on the crops. Similar arguments may be raised for and against the use of genetically engineered products to increase productivity of farm animals.

Medicines The production of medical products by genetically engineered organisms is generally less controversial than the production of foods. Some possible reasons for this are the fact that the medicines may be considered more essential than modified versions of existing foods, that production occurs in specialised units rather than the open environment, and that this branch of genetic engineering mainly involves microorganisms which do not raise the same ethical issues as other organisms. Production of antibiotics, insulin, factor VIII (a blood clotting enzyme), and growth hormone by genetically engineered microorganisms have had immeasurable benefits to health. These substances are more effective than former products, carry fewer health risks, and in the case of insulin, prevent the killing of animals to obtain the product. Another major benefit is that once production is running it is very cheap to produce these substances in fermenters, meaning that they can be used in developing countries as well as developed.

Genetic engineering of mammals for the production of alpha-1-antitrypsin and other products such as factor IX (a blood clotting enzyme) has raised less media attention than might be expected, presumably because such work is mostly at the research stage. As with all uses of animals for research there are animal rights issues related to the well-being and care of the animals which have been genetically modified. Measures are in place in Britain to protect transgenic animals from undue suffering in the same way as other laboratory animals are protected. Yet some groups still argue that it is unethical to use animals at all. From the human point of view one possible cause for concern is the hazard of contamination of animal products with diseases (such as BSE).

Gene therapy, a type of genetic engineering in humans raises many issues. Whilst the use of somatic gene therapy in treating life threatening diseases such as cystic fibrosis may be considered beneficial, many people fear that the techniques developed here could be extended to other situations. For example, in the future it might be technically possible to manipulate the genes of an unborn child to reverse mild medical conditions, and alter appearance or personality. At present research into germ line gene therapy, the form which would alter the genes of all cells including the gametes, is banned in Britain. Any developments in this area must balance improvements in health of the population and potential savings in the cost of treating genetic diseases with expensive medications, against the threats to human identity and diversity.

3 Physiology and Transport

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3.1

Transport systems

Content:

Mass transport

- ▼ The bulk movement of substances through transport systems.
- ▼ The transport systems of larger organisms and their links with specialised exchange systems whose main function is to maintain concentration gradients.

Mammalian heart

- ▼ The structure and function of the heart, including the atria and the ventricles, atrioventricular and semilunar valves.
- ▼ The cardiac cycle

Blood vessels

- ▼ The structure of arteries, arterioles, veins and capillaries related to their functions.

Exchange of materials

- ▼ The main substances transported by the blood stream, and the sites at which exchange occurs.
- ▼ Oxygen uptake and dissociation by haemoglobin
- ▼ The effects of pH and carbon dioxide concentration on the oxyhaemoglobin dissociation curve.

Tissue fluid

- ▼ The relationship between blood, tissue fluid, lymph and plasma
- ▼ The role of the lymph system in the return of tissue fluid to the blood system.

MASS TRANSPORT

All large multicellular organisms above a certain level of complexity, require transport systems. Over large distances in organisms, efficient supply of materials is provided by mass transport (the bulk movement of substances through transport systems). The transport systems of larger organisms are often intimately linked with specialised exchange systems, whose main function is to maintain concentration gradients between the transport fluid (e.g. blood) and the environment. Materials exchanged between the transport fluid and the environment, and the transport fluid and the cells include, respiratory gases, nutrients, waste products and in the case of plants gases involved in photosynthesis.

In animals with a transport system, the blood is circulated around the body by mechanical pumping of a heart and contractile blood vessels. Diffusion is however still important in transport, as it is by diffusion through large surface areas that substances enter and leave the blood, for example at the lungs and at the surface of the small intestine in mammals, and between the capillary beds and the tissues.

In plants with a transport system, water is moved by forces generated by evaporation, and organic substances by pressure gradients based on water potential.

MAMMALIAN HEART

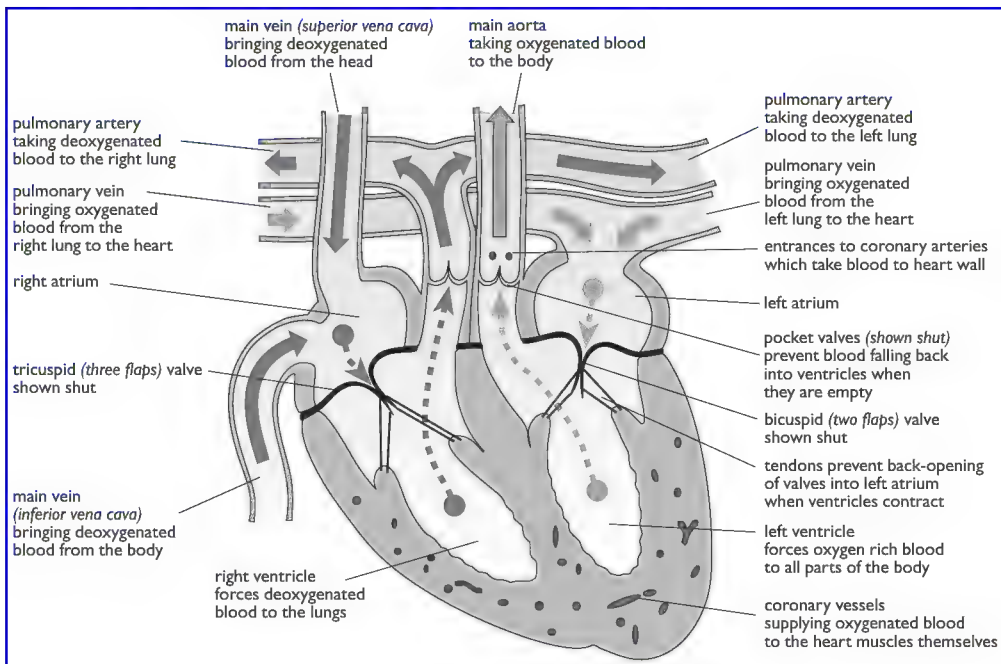
Structure and function

The heart is situated slightly to the left of the midline of the chest, protected by the ribs and by membranes (pericardial membranes) which surrounds it in a fluid filled pericardial cavity.

It is composed of four chambers, two upper thinner walled atria, and two lower thicker walled ventricles. The right and left sides are divided from each other by a thick septum. The right atrium receives blood from the venae cavae from the body, and the left atrium receives blood from the pulmonary veins from the lungs. (Note that diagrams of internal organs are always viewed as from the front of the body, so that the right atrium and right ventricle appear on the left side of the diagram.)

◆ CHECKPOINT SUMMARY

- ◆ Surface area increases with size but surface area to volume ratio decreases
- ◆ SA/V increased by flattened body shapes and structures
- ◆ Where SA/V sufficiently large, simple diffusion suffices for exchanges with environment and internal transport
- ◆ Discussions of development of transport systems also involve level of complexity of organism
- ◆ Over large distances in organisms, efficient supply of materials is provided by mass transport (the bulk movement of substances through transport systems)
- ◆ Development of transport systems in animals involves pumping hearts and tubes
- ◆ The transport systems of larger organisms involves development of specialised exchange surfaces with large surface areas
- ◆ Specialised exchange surfaces in flowering plants are the leaves and roots, and in the mammal the lungs, gut, and kidneys.



The atria are separated from the main pumping chambers, the right and left ventricles, by flaps of fibrous tissue which act as one way valves. These **atrioventricular valves** differ from each other. The **tricuspid** valve on the right has three flaps, and the **bicuspid** valve on the left, two. These are attached to tendons and muscles which allow them to be forced shut, but prevent them opening upwards and allowing blood back into the atria, when the pressure in the ventricles increases as they contract.

Inside the entrance of the main arteries which leave the heart, the aorta and pulmonary artery, are two more sets of valves called semi-lunar or pocket valves, which prevent the back flow of blood into the ventricles when they relax by filling with blood and blocking these main arteries. Immediately above the semi-lunar valve in the aorta is the entrance to the **coronary artery** which supplies the heart itself with nutrients and oxygen. This spreads its branches all over the surface of the heart. Deoxygenated blood is collected into the coronary vein which empties directly into the right atrium. The heart requires its own blood supply despite being filled with blood, as the blood that it is pumping is passing through too quickly and the heart walls are too thick for diffusion to supply the needs of the heart muscle fibres.

The walls of the atria are less muscular than those of the ventricles, and the wall of the left ventricle is more muscular than that of the right ventricle. These differences reflect the different pressures that need to be generated in the chambers. The atria only have to pump blood into the ventricles. The right ventricle pumps blood to the lungs at a relatively low pressure. The lungs are close to the heart, and too high a pressure could damage the delicate alveoli and result in the filling of the lungs with fluid. The left ventricle must generate a high pressure to pump blood all around the body.

However, it is important to remember that the volumes of the cavities of the chambers on the right side of the heart are the same as those on the left side of the heart, otherwise the right side would not be able to supply the left side with sufficient blood.

The events of the cardiac cycle

The sequence of events that occurs during the filling and emptying of the heart is known as the cardiac cycle. At a rate of 70 beats per minute (bpm) the complete cycle takes about 0.86 seconds. It is a continuous sequence of events that occurs simultaneously on the left and right sides, but for the purposes of explanation it is convenient to consider it as occurring in a series of stages

- ▼ There is a point in the cardiac cycle when all the heart valves are shut, which can be taken as the starting point of the cardiac cycle.
- ▼ Blood returning under low pressure in the main veins (superior and inferior venae cavae) from the upper and lower body enters the right atrium, and at the same time blood returning from the lungs in the pulmonary veins enters the left atrium.
- ▼ The atria fill with blood, and the rising pressure of the blood in the atria pushes open the tricuspid and bicuspid valves, and both ventricles begin to fill, increasing the internal volume and pressure.
- ▼ As the heart fills with blood both atria contract (**atrial systole**) forcing even more blood into the ventricles. These are now stretched, and they both contract (**ventricular systole**)

◆ CHECKPOINT SUMMARY

- ◆ External appearance of heart includes small 'flap-like' atria, coronary blood vessels, fat deposits, and major vessels entering and leaving
- ◆ Internal structure of two atria and two ventricles, with atria and ventricles separated by bicuspid and tricuspid valves, and the left and right sides separated by the septum
- ◆ Chordae tendinae and muscles prevent back-opening of bicuspid and tricuspid valves (atrioventricular valves) when ventricles contract (ventricular systole)
- ◆ Pocket (semilunar) valves at base of pulmonary artery and main aorta prevent backflow of blood into ventricles when they relax (ventricular diastole)
- ◆ Atria walls thinner than ventricle walls.
- ◆ Left ventricle wall thicker than right ventricle wall to pump blood around body (systemic circulation)
- ◆ Right ventricle wall thinner as only has to pump blood via pulmonary circulation around lungs, where reduced pressure prevents formation of tissue fluid in alveoli
- ◆ Volumes of both left and right ventricles the same, as right ventricle supplies the left ventricle with blood via the lungs and left atrium.

decreasing the internal volume, increasing the internal pressure, and forcing the blood upwards.

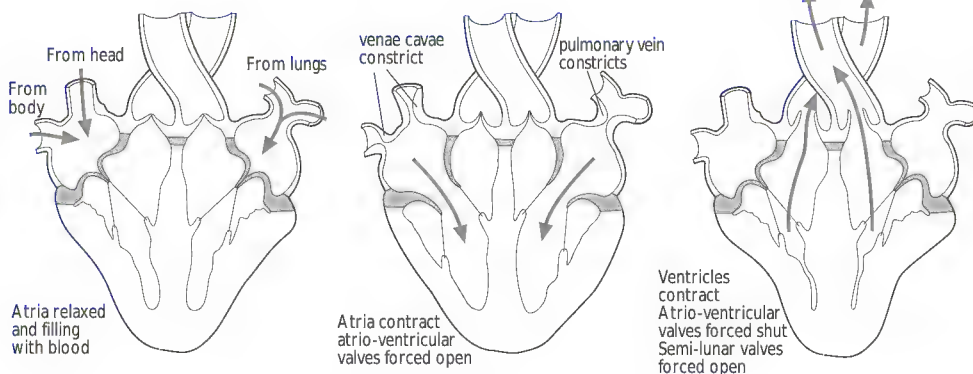
- ▼ This upsurge of blood forces the tricuspid and bicuspid valves shut. These valves are prevented from back-opening into the atria by tough tendon cords, which are held taut by contraction of the papillary muscles to which they are attached. The closing of these valves makes the first heart sound (described as 'lub').
- ▼ The blood is thus forced along the pulmonary artery to the lungs, and along the main aorta to the rest of the body, forcing open both sets of pocket or semi-lunar valves on the way.
- ▼ When the ventricles relax (**ventricular diastole**) their internal pressure drops, and the blood tends to fall back down the pulmonary artery and main aorta under gravity, thus filling and shutting the pocket valves. The closing of these valves makes the second heart sound (described as 'dup'). All valves are now shut and the cycle is repeated.

The heart sounds can be heard with the use of a stethoscope.

◆ CHECKPOINT SUMMARY

- ◆ The events of a complete filling and emptying of the heart is known as the cardiac cycle
- ◆ It is a continuous cycle, start point of which can be taken when heart is empty and all valves shut
- ◆ Atria fill and internal pressure rises forcing bicuspid and tricuspid valves open
- ◆ Ventricles fill so that whole heart full.
- ◆ Atria contract (atrial systole) so that ventricles overfilled and stretched
- ◆ Ventricles contract with a force proportional to their degree of stretch (ventricular systole)
- ◆ Blood forced up shutting bicuspid and tricuspid valves (first heart sound) and opening pocket valves as blood forced up pulmonary artery and aorta
- ◆ Ventricles relax (ventricular diastole) blood falls back in pulmonary artery and aorta shutting pocket valves (second heart sound)
- ◆ During systole the pressure inside the heart and the arteries rises (systolic blood pressure)
- ◆ During diastole the pressure inside the heart and the arteries falls (diastolic blood pressure)
- ◆ These waves of pressure are felt as the pulse in the arteries.

Cardiac Cycle



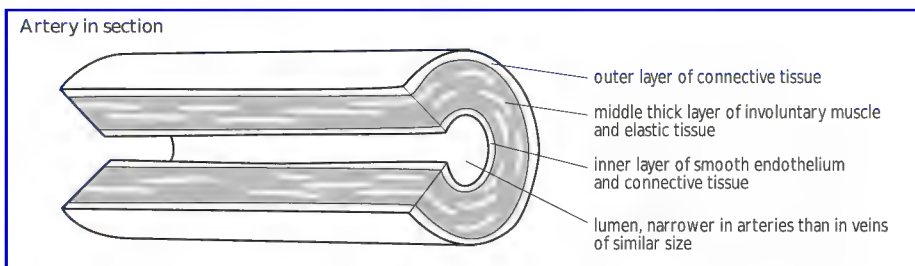
BLOOD VESSELS

In mammals the blood circulates around the body in a continuous system of blood vessels. From the heart, blood travels in the arteries and arterioles, into the capillaries supplying the tissues, and then returns to the heart in the venules and veins. All blood vessels are lined with smooth endothelium (epithelium found lining tubes) which reduces friction. Arteries and veins have varying amounts of muscle in their walls, which is always involuntary (smooth / unstriated) muscle under the control of the autonomic nervous system and hormones.

Arteries

Arteries close to the heart have a large cross sectional area, and thick elastic walls. Arteries are stretched by the cardiac output when the heart contracts, and they recoil when the arterial blood pressure drops as a result of the heart relaxing between beats. The recoil of the elastic artery walls in this way assists the circulation of the blood around the body. It helps smooth the flow between contractions of the heart, and helps to maintain the blood pressure. This stretching and recoiling of the arteries is felt as the pulse in places where arteries come close to the surface of the body (the inside of the wrist, the side of the neck, and in the temple).

The main artery leaving the left ventricle of the heart, the aorta, branches into arteries supplying all the main regions of the body. Further from the heart, there is a gradual transition from the elastic arteries to muscular arteries that have circular layers of involuntary muscle in their walls. These arteries branch into smaller **arterioles** the walls of which, although thinner, also have circular layers of smooth muscle. The state of contraction or 'tone' of these muscle layers alters the diameter of these vessels, which alters the resistance to the flow of the blood, which in turn affects the distribution of the blood to different parts of the body and the blood pressure. The arterioles penetrate all tissues of the body and connect to beds of finely branching capillaries.



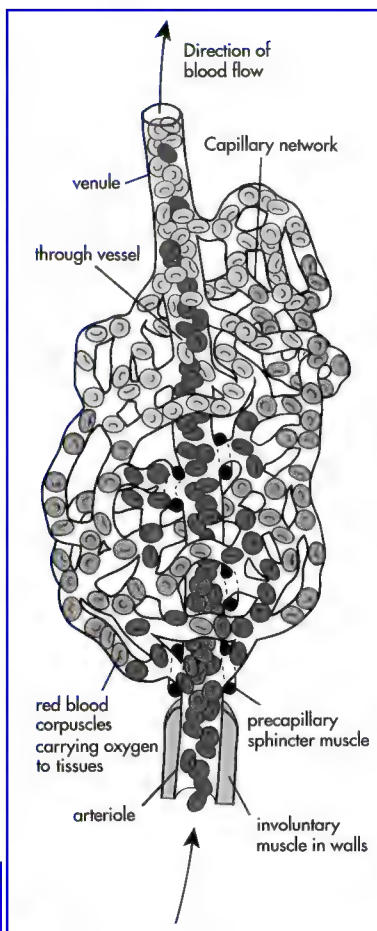
Capillaries

Capillaries form a fine network which penetrate all tissues. They have the smallest diameter of all blood vessels, about $6-8\text{ }\mu\text{m}$ (0.007 mm), which is the same as that of the red blood cells. For this reason the red cells are forced to move slowly through the capillaries in single file, ensuring a short diffusion distance between the corpuscles and the tissues. Capillary walls are just one cell thick, which further aids exchanges by diffusion between the blood and the tissues. The huge number of capillaries provide a very large total surface area across which these exchanges occur. As more capillaries open up in working muscles, the surface area between the blood and tissues is increased, and the diffusion distance between the two is decreased, resulting in more rapid and efficient exchanges between them. The blood pressure and rate of flow both drop in the capillaries. The slow flow also increases the time available for exchange of materials between the blood and the tissues.

There are so many capillaries in the body that they cannot all be supplied with blood at the same time. Consequently, there is competition for blood between different regions of the body, especially during exercise, when blood must be shunted to the working muscles, and consequently withdrawn from other regions. For example, when the body is at rest, about 45% of the cardiac output passes through the capillaries of the gut wall and associated glands, and the kidneys. During maximum exercise this can be reduced to about 3% of the cardiac output, as blood is redirected to the working muscles. The reduced flow rate is compensated for by a greater extraction of oxygen from the blood, by these tissues. The shunting of blood between competing tissues is achieved by constriction and dilation of the arterioles, and of the arterio-venous vessels, which are direct connections between the arterioles and venules. The entrances to the capillary beds are controlled by circular precapillary sphincter muscles which, when contracted, shut off the capillaries and when relaxed, open them.

Estimated blood flow in cm^3 per minute, to different organs/systems in a trained male at rest and during maximum effort.

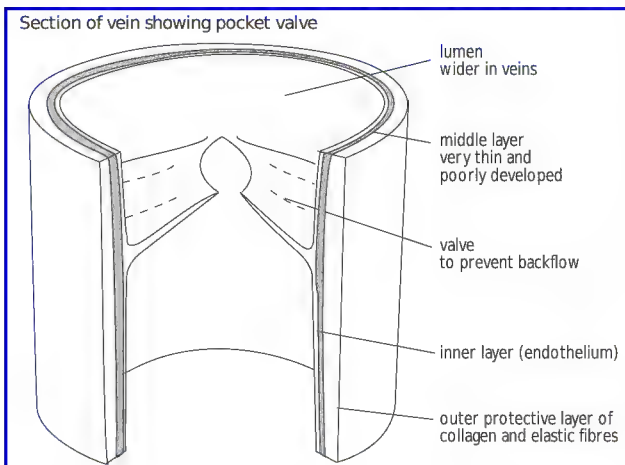
Organ system	At rest	%	Max. effort	%
Skeletal muscle	1000	20	26 000	88.00
Coronary vessels	250	5	1200	4.00
Skin	500	10	750	2.50
Kidneys	1000	20	300	1.00
Liver & gut	1250	25	375	1.25
Brain	750	15	750	2.50
Whole body	5000	100	30 000	100.00



Veins

Capillaries join up to form **venules**, which in turn join to form veins which return the blood to the heart. The individual veins have a larger cross sectional area than the comparable arteries, and as there are more veins than arteries, the veins also have a greater total cross sectional area. The walls of the veins are thinner and less elastic than those of the arteries, and in the veins of the legs and arms there are semi-lunar or pocket valves at intervals along their length. These valves oppose any back flow of blood under gravity, and ensure one-way flow of blood to the heart when the veins are 'massaged' by movement of surrounding tissues (especially contracting skeletal muscles). The thin walled veins with their large cross section present little resistance to the flow of the blood, so that although the blood pressure in the veins is low after passage of the blood through the capillary beds, the remaining pressure still helps move the blood in the veins back to the heart.

Because the blood in the veins is under low pressure, and their walls are thin, the veins are easily compressed by the smallest movements of the surrounding structures, particularly the skeletal muscles. As the muscles contract and relax during exercise they exert a massaging effect on the deep veins, especially for example in the legs, when running, swimming, or cycling. This massage effect of the 'muscle pump' is random and in no particular direction, but the pocket valves in these veins ensure that the flow is one-way, back to the heart.



In addition, breathing movements assist the return of blood in the veins to the heart. On breathing in, the diaphragm contracts and moves downwards. This decreases the pressure within the thorax, and increases the pressure within the abdomen. These changes in pressure compress the large veins in the abdomen, and assist the flow of blood into the veins of the thorax which return blood to the heart.

At rest the veins act as a large reservoir of blood for use when circulatory demands increase, for example during exercise. During exercise when the output of blood from the heart (cardiac output) increases, the muscle fibres in the walls of the veins are stimulated to contract (venous tone) and the veins constrict. As a result a larger

◆ CHECKPOINT SUMMARY

- ◆ Arteries thick elastic walls with smooth muscle.
- ◆ Elasticity of arteries important in smoothing flow of cardiac output - the pulse
- ◆ Lined with smooth endothelium common to all blood vessels.
- ◆ Dividing into smaller branches leading into arterioles
- ◆ Capillary beds supply all tissues providing large surface areas for exchanges by diffusion and short diffusion distances.
- ◆ Capillaries have large total cross sectional surface areas. Therefore resistance to flow, blood pressure and rate of flow all drop in capillaries.
- ◆ Diameter of capillary same as red blood corpuscles thus forcing them to pass through capillaries in single file, bringing all close to tissues.
- ◆ Plasma minus proteins exuded through thin walls to bathe tissues directly.
- ◆ Thin walls and large lumen of veins offer little resistance to flow of blood back to heart, and allow ease of massage by surrounding muscles.
- ◆ Pocket valves ensure one way flow back to heart.
- ◆ Venous blood under low pressure but flow fast enough to ensure return of blood to heart at same rate as blood flow in the arteries.

proportion of the venous blood is shunted back to the heart, especially from the veins in the legs and the abdomen. This is especially important in preventing blood from 'pooling' in the legs under the force of gravity during upright exercise, and immediately afterwards.

In these ways the venous return of blood balances the rising cardiac output during exercise. This is essential as the heart would be unable to maintain a high cardiac output unless it was receiving an equal volume of blood back from the veins.

EXCHANGE OF MATERIALS

The main substances transported by the blood stream with regard to the exchange of materials are nutrients, waste products, heat and respiratory gases

Nutrients The soluble end products of digestion, glucose, amino acids, fatty acids and glycerol; plus water, inorganic ions and water soluble vitamins are absorbed in the intestine into the blood plasma. Lipid compounds and fat soluble vitamins are transported as tiny globules in the lymphatic system, or combined with blood proteins in the plasma. The blood draining from the gut travels first to the liver which regulates the levels of many substances in the blood, before it passes around the body.

Waste products Other than carbon dioxide (described below) the main waste product of metabolism is urea which is formed in the liver as a breakdown product of excess amino acids which cannot be stored by the body. Urea is excreted at a specialised exchange surface in the kidney, filtering out of the blood under pressure into the kidney tubules. The kidney also adjusts the water and inorganic ion content of the blood.

Oxygen and carbon dioxide Oxygen and carbon dioxide are exchanged between the cells and the blood and tissue fluid; and between the blood and the air in the alveoli of the lungs. At the lungs carbon dioxide is released from the blood, and oxygen taken up; and at the tissues oxygen leaves the blood and carbon dioxide is taken up.

Oxygen absorbed into the blood in the alveolar capillaries combines with haemoglobin in the red blood cells to form **oxyhaemoglobin**. Each molecule of mammalian haemoglobin consists of four sub-units, each consisting of an iron-containing haem group combined with a protein globin part. Each subunit combines reversibly with one molecule of oxygen. Thus one molecule of haemoglobin can carry four molecules of oxygen. The formation of oxyhaemoglobin from deoxyhaemoglobin involves a physical holding of the oxygen by the haemoglobin sub-units as a result of their special 3-dimensional structure. In man each red blood cell contains about 300 000 000 molecules of haemoglobin. At rest and even during maximum exercise, at sea level, the haemoglobin in the blood leaving the lungs in healthy subjects is virtually saturated with oxygen. Therefore the capacity and functioning of the lungs is not normally the limiting factor in exercise.

The oxygen is easily displaced by carbon monoxide, which combines irreversibly with haemoglobin to form carboxyhaemoglobin. Major sources of carbon monoxide are smoking and car exhaust fumes.

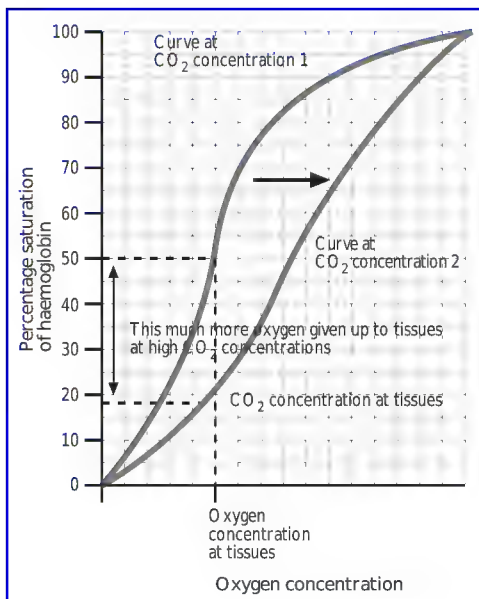
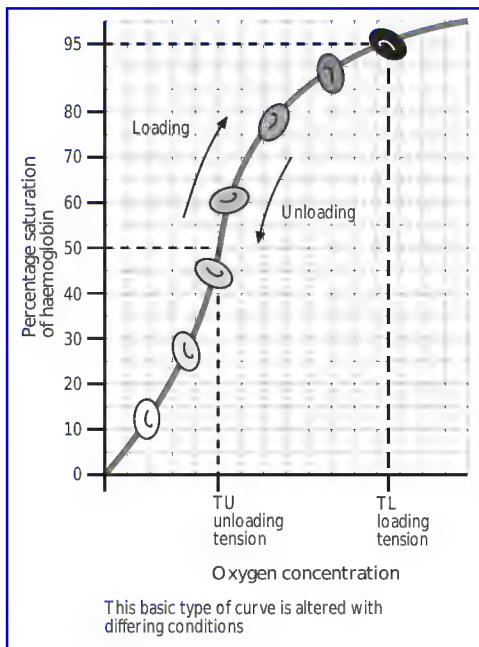
Gas exchange at the tissues is the opposite to that at the lungs with oxygen being released and carbon dioxide taken up. Tissues vary

widely in their demand for oxygen and in their production of carbon dioxide, particularly during exercise when the muscles become more active. The low oxygen partial pressure in the muscles during exercise increases the diffusion gradient between them and the red blood corpuscles, thus encouraging the dissociation of the oxyhaemoglobin and the release of oxygen in solution to diffuse to the tissues.

Some carbon dioxide is carried in the red blood cells in combination with haemoglobin as **carbaminohaemoglobin**. Some carbon dioxide is carried in the plasma in simple solution, but most is carried as hydrogen carbonate ions in the plasma. The formation of these is greatly accelerated as a result of the action of an enzyme (**carbonic anhydrase**) within the red blood cells. Carbon dioxide released as a waste product of cellular respiration dissolves in the plasma to form carbonic acid. This enters the red blood cells where the enzyme catalyses its dissociation into hydrogen ions and hydrogen carbonate ions. The hydrogen ions accelerate the dissociation of oxyhaemoglobin to release oxygen to the tissues, and the hydrogen carbonate ions enter the plasma to be carried to the lungs. The release of hydrogen carbonate ions into the plasma from the red blood corpuscles is balanced by the uptake of chloride ions from the plasma (the chloride shift). When the blood reaches the alveolar capillaries these events are reversed, hydrogen carbonate ions enter the red blood corpuscles, carbon dioxide is released, and chloride ions return to the plasma.

The effects of pH and carbon dioxide concentration on oxygen transport by haemoglobin

As has been described the red blood cells are involved in both oxygen and carbon dioxide exchanges and transport. At the tissues, the carbon dioxide, produced as a waste product of aerobic respiration in the mitochondria, diffuses in solution down its concentration gradient, from the high concentration (partial pressure - $p\text{CO}_2$) in the mitochondria to the lower $p\text{CO}_2$ in the blood. The greater the difference in $p\text{CO}_2$ the greater the amount of diffusion. The formation of carbonic acid (and lactic acid in anaerobic respiration) lowers the pH of the blood. Any increase in acidity (decrease in pH) and/or increase in temperature encourages oxyhaemoglobin to release its oxygen to the tissues. The way in which oxygen is held and released by haemoglobin at different partial pressures can be represented by an oxygen association/dissociation curve. An increased tendency of oxyhaemoglobin to release its oxygen is shown by the oxygen association/dissociation curve shifting to the right. This effect is called the **Bohr shift** and its significance is that the more active the tissue (e.g. muscle), the more carbon dioxide, lactic acid, and heat are produced and the more oxygen is released to that tissue.



◆ CHECKPOINT SUMMARY

- ◆ The main substances transported by the blood system, and the sites at which exchange occurs relate to respiration, nutrition and excretion
- ◆ Oxygen combines reversibly with haemoglobin to form oxyhaemoglobin in which form it is transported from the alveoli to the tissues
- ◆ Haemoglobin loads up with oxygen at the alveoli, at the tissues higher temperatures, and high levels of carbon dioxide and lactate which lower pH encourage the dissociation of oxyhaemoglobin and the release of oxygen to the tissues
- ◆ These events are illustrated in an oxygen haemoglobin dissociation curve which is moved to the right as the affinity for oxygen decreases
- ◆ Carbon dioxide is transported in the blood as carbaminohaemoglobin in the red blood cells and hydrogencarbonate ions in the plasma to be released into the alveoli
- ◆ The soluble end products of digestion: glucose, fructose, amino acids, glycerol and fatty acids are absorbed from the gut and transported to the liver where exchanges occur to optimise the blood plasma composition
- ◆ Waste products e.g. urea are collected from the liver, transported to the kidneys where osmoregulation and excretion are carried out, with urea being excreted and the blood concentration being optimised.

TISSUE FLUID

The relationship between blood, tissue fluid, lymph and plasma

Blood consists of the fluid plasma, which carries red blood cells, white cells, and fragments of cells from the red bone marrow known as platelets.

Plasma consists of 90% water, and 10% materials in suspension and solution. Plasma proteins, formed mainly in the liver, include albumin (to which the plasma calcium is bound), globulins (of which some are antibodies and some are important in the transport of hormones and vitamins), and fibrinogen (which is important in blood clotting). These proteins have an important function in buffering the blood (preventing sudden changes in acidity and alkalinity). They are also involved in regulating the movement of water between blood and tissues, including preventing the accumulation of fluid in the tissues. Plasma also transports many inorganic and organic materials e.g. amino acids, glucose, fats, urea, creatine, hydrogencarbonate, some oxygen and carbon dioxide. The characteristic yellow straw colour comes from a yellow pigment molecule called bilirubin which is formed from haemoglobin when old red blood cells are broken down in the liver and spleen.

Red blood cells lose their nucleus and most of their cytoplasmic organelles during their maturation in the red bone marrow. They become 'corpuscles' with a flexible membrane, filled with the oxygen carrying pigment haemoglobin. The loss of the nucleus accounts for their biconcave shape which increases their surface area to volume ratio. The total surface area of the red blood cells in man is about 3500 m² and this facilitates the exchange of gases between the red cells and the tissues. The biconcave shape also gives them some resilience in absorbing water without bursting should the plasma become temporarily diluted for any reason. The flexible membrane enables red cells to 'squeeze' through capillaries.

Red blood cells comprise about 30-40% of the blood volume.

White cells, of which there are two main types (phagocytes and lymphocytes), form the basis of the immune system and are described in detail in section 5.2.6. Together with platelets they comprise just 1% of the total blood volume.

Platelets are cell fragments, which have an important function in the clotting process.

Tissue fluid

Tissue fluid is formed when plasma, minus its proteins, is pushed out under pressure through the capillary walls to bathe the tissues. Depending on the degree of activity and health and fitness, most of the tissue fluid is reabsorbed back into the blood capillaries. Tissue fluid forms the internal environment of multicellular animals. It bathes each cell and is the medium for the exchange of materials between the individual cells and the blood system.

Lymph

Lymph is that part of the tissue fluid which is not reabsorbed by the blood capillaries, and enters the lymphatic capillaries (which are found in all tissues) of the lymphatic system. The blind-ended lymphatic capillaries lead into wider lymph vessels which are similar in structure to the veins, having thin walls with pocket valves. Contraction of skeletal muscles massages the vessels and the valves ensure unidirectional flow of the lymph back to the major veins near the heart. This in turn aids the draining of tissue fluids.

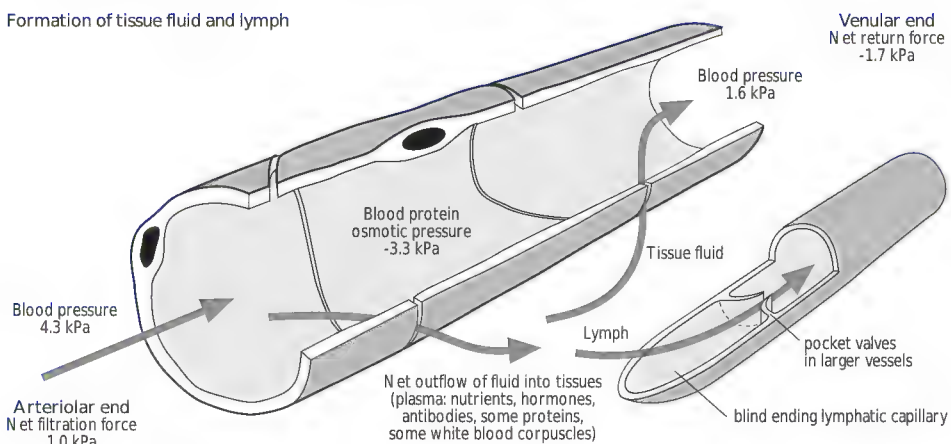
During exercise the blood pressure may increase, more tissue fluid is exuded, the lymph vessels are massaged more vigorously, and therefore the tissue fluid is drained more quickly. All these factors increase the speed of supply of oxygen and nutrients to, and removal of waste products from, the tissues.

At intervals in the lymphatic system are swellings called lymph nodes, which contain phagocytes and lymphocytes and play an important role in the defence of the body against infection.

◆ CHECKPOINT SUMMARY

- ◆ The hydrostatic pressure of the blood in the capillaries causes plasma minus its proteins to be forced through the thin walls of the capillaries
- ◆ This fluid bathes all the tissues and is known as tissue fluid
- ◆ It carries substances in solution from the blood to supply the tissues, e.g. oxygen, nutrients, hormones, antibodies etc; and drains waste products e.g. carbon dioxide away
- ◆ Most of the tissue fluid is reabsorbed back into the capillaries as the hydrostatic pressure drops as the blood passes through the capillaries
- ◆ Excess tissue fluid is drained away into blind ending lymphatic capillaries.
- ◆ Lymph is tissue fluid that has been drained into lymphatic capillaries, with lymphocytes added by lymph nodes which occur at intervals
- ◆ Special lymph capillaries in the villi of the lining of the small intestine, known as lacteals, preferentially absorb and transport fats, so that lymph contains more fats than plasma
- ◆ Eventually the lymph is returned to the general circulation in the major veins near to the heart
- ◆ Movement of the lymph in the lymphatic system is maintained by the massaging effects of the muscles and breathing movements, and the one-way pocket valves; all features common to the veins of the circulatory system.

Formation of tissue fluid and lymph



3.2

The control of breathing & heart rate

Content:

Control of ventilation

- ▼ The role of the medulla in the brain and of the stretch receptors in the lungs in the maintenance of breathing.
- ▼ The role of the medulla in the brain and of the receptors in the lungs, aorta and carotid bodies in the response of the breathing system to increased muscular activity.

Control of heartbeat

- ▼ The role of the sinoatrial node, the atrioventricular node and the bundle of His in the maintenance of heartbeat.
- ▼ The role of the medulla, pressure receptors and chemoreceptors in the walls of the aorta and carotid sinuses in the response of the heart to increased muscular activity.

CONTROL OF VENTILATION

Breathing movements are under nervous control.

Normal breathing movements are controlled by the involuntary, automatic, rhythmic discharges of nerve impulses from areas of the **medulla** of the brain known as the **respiratory control centres**.

The **inspiratory control centre** sends motor nerve impulses to the external intercostal muscles of the ribs, and the muscles of the diaphragm, causing them to contract, raising the ribs and flattening the diaphragm, and inspiration to occur. This active phase of the inspiratory control centre lasts for about two seconds. Then it automatically stops sending motor impulses for about three seconds, during which time the intercostal muscles of the ribs, and the muscles of the diaphragm relax, and expiration follows passively.

The basic rhythm of nervous control by the respiratory control centres is influenced by sensory inputs so that breathing alters in response to the demands of the body during exercise.

During forced breathing, when the inspiratory control centre reaches the end of each of its periods of rhythmic discharge, it sends nerve impulses to activate the **expiratory control centre**. Motor impulses from here stimulate the internal intercostal muscles and abdominal muscles to contract, resulting in forced expiration.

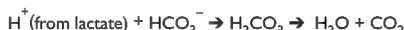
Ventilation of the lungs is a function of the **rate** and **depth** of breathing. Early in exercise, ventilation is mainly increased by an increase in the depth of breathing, whilst later it is the rate of breathing that increases more.

Any increase in the acidity of the blood (decrease in blood pH) causes an increase in ventilation. A decrease in pH results mainly from an increase in the production of **lactate** (see 12.3) and carbon dioxide,

due to the increase in the rate of respiration associated with exercise. The level of acidity is monitored by **chemoreceptors** in the main aorta and in the carotid bodies in the arteries in the neck. Indeed, the sensitivity of the carotid bodies to a decrease in pH is thought to be a major factor controlling the increase in ventilation during exercise, along with nervous stimulation of the respiratory control centres from the contracting muscles and moving joints.

The respiratory control centres have connections with the cerebral cortex (conscious centres), so that a degree of voluntary control can be exerted over breathing movements. This is necessary for speech, and to exclude water from the lungs, as would be the case in swimming. However, once the pH decreases below, and the carbon dioxide in the blood rises above, a certain level, the inspiratory control centre is stimulated, and voluntary control is over-ridden. It is these factors rather than shortage of oxygen that limit breath holding activities.

The blood has a certain buffering capacity; that is under normal conditions it can resist changes in its pH so that the blood pH only varies between 7.2 and 7.45. During exercise as the lactate accumulates in the blood, it is buffered (initially at least) by combining with hydrogen carbonate ions present in the plasma, so increasing the amount of carbon dioxide in the blood.



An increase in the level of CO_2 in the blood stimulates the chemoreceptors in the carotid bodies, but its main effect is via direct stimulation of the respiratory control centres in the brain, resulting in an increase in the ventilation rate, and therefore more CO_2 being exhaled by the lungs.

Past a certain point the buffering capacity of the blood (due also to plasma proteins and haemoglobin) is saturated, and due to the accumulation of lactate in strenuous exercise the pH within the muscles can drop to 6.4, and the arterial blood pH to about 7.0 or just below, as a result of the accumulation of lactate. Typically the ventilation of the lungs during exercise increases in proportion to the increase in heart rate and to the uptake of oxygen, however as the level of lactate reaches 4 millimoles per litre of blood the rate of ventilation of the lungs increases more rapidly than the heart rate and the oxygen uptake. This point, at which ventilation 'breaks away' from the heart rate and oxygen uptake, is known as the '**ventilation break point**'. Past this point, although the rate of ventilation increases, there is no further increase in the uptake of oxygen. This point is also known as the '**anaerobic threshold**' (see 12.3).

The chemoreceptors in the carotid bodies and the aorta are also sensitive to levels of oxygen in the blood, although less so than to the levels of carbon dioxide and pH. A decrease in the level of oxygen stimulates an increase in ventilation.

Stretch receptors in the bronchi and the bronchioles are stimulated by the expansion of the lungs. Nerve impulses pass from these along sensory nerves to the inspiratory control centre, causing the reflex inhibition of the inspiratory motor nerve impulses. Therefore the intercostal muscles and diaphragm relax, and expiration follows passively. However, this mechanism is not thought to be part of the normal control of breathing, but operates to protect the lungs from over-inflation. Other sensory receptors (proprioceptors) in the skeleton and muscles, also feedback sensory information to increase breathing movements during exercise.

◆ CHECKPOINT SUMMARY

- ◆ For efficient gaseous exchange at the alveolar surface, air must be moved in and out of the lungs in the process called ventilation
- ◆ Inspiration (breathing in) - the external intercostal muscles contract, pulling the ribs upwards and outwards, the diaphragm contracts, moving downwards, the volume of the thorax increases, the pressure in the thorax decreases below atmospheric pressure causing air to enter; inflating the lungs
- ◆ Expiration (breathing out) depends upon the elasticity of the tissues of the lungs and thorax. The respiratory muscles relax, the thorax and lungs recoil as a result of their elasticity and the events of inspiration are reversed
- ◆ Normally, respiratory movements are involuntary, being controlled by rhythmic discharges of nerve impulses from the respiratory control centres in the medulla region of the brain
- ◆ Chemoreceptors in the aortic bodies and carotid bodies detect rises in carbon dioxide concentration and decreases in pH and relay impulses to the control centres in the medulla of the brain, resulting in increased rate and depth of breathing
- ◆ From the inspiratory control centre impulses travel down the phrenic nerves to the diaphragm, and down the intercostal nerves to the external intercostal muscles causing them to contract and bring about inspiration
- ◆ At the end of inspiration, stretch receptors in the bronchioles send impulses to the inspiratory control centre in the medulla, which is switched off, and the diaphragm and external intercostal muscles relax. The elasticity of the tissues of the lungs and thorax leads to expiration as they recoil.

In conclusion it should be noted that the changes in blood levels of carbon dioxide, pH, and oxygen, together with mechanical feedback, cannot fully account for the large and rapid changes in ventilation experienced during exercise. Other factors increasing ventilation, include a rise in body temperature, and an increase in the blood levels of adrenaline and/or noradrenaline. The emotions are also involved, possibly via the sympathetic nervous system and the adrenal glands. Their involvement may help explain the anticipatory rise in ventilation that can occur before the start of exercise.

CONTROL OF HEARTBEAT

Cardiac muscle is myogenic, that is the stimulus for contraction arises internally, rather than externally from the nervous system, as occurs with skeletal muscle.

Sinoatrial node

The sinoatrial node (SA node) or 'pacemaker' is a mass of specialised tissue in the right atrium from which coordinating waves of electrical activity originate and radiate through the muscle of the heart wall. The sinoatrial node thus coordinates the basic rhythm of the heart contractions.

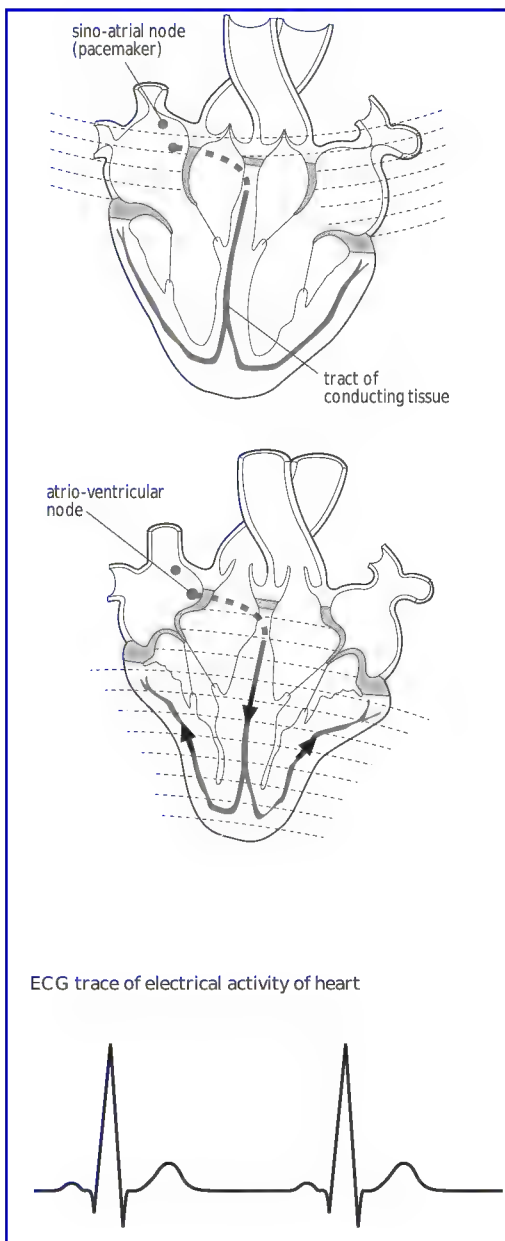
The wave of excitation spreads rapidly through the interconnected cardiac muscle fibres of the atria resulting in both contracting simultaneously. A band of fibrous connective tissue between the atria and ventricles prevents the wave of excitation spreading down into the ventricles.

Atrio-ventricular node

Another mass of specialised tissue, the atrio-ventricular node at the bottom of the atria is stimulated by the waves of electrical impulses radiating from the SA node. It transmits the impulses through the insulating band of connective tissue between the atria and ventricles. The impulses travel to the base of the ventricles in a tract of conducting tissue, composed of modified cardiac muscle fibres and neurones known as Purkinje tissue, from where the impulses radiate upwards through the cardiac muscle of the ventricles.

This pattern of the spread of excitatory waves through the heart, ensures that the atria contract first to force the blood **down** into the ventricles, and that the ventricles subsequently contract to force the blood **up** into the pulmonary arteries and main aorta. The spread of the wave of excitation throughout the heart can be detected by electrodes attached to the skin of the chest and displayed as an electrocardiograph (**ECG** trace).

The onset of strenuous activity can result in ECG abnormalities due to insufficient blood flow to the heart muscle in the first few seconds. This can be prevented by a two minute warm up period before exercise.



◆ CHECKPOINT SUMMARY

- ◆ Cardiac muscle is myogenic
- ◆ Rate modified by sino-atrial node (pacemaker) in wall of right atrium
- ◆ Waves of electrical activity spread rapidly down through cardiac muscle of atria
- ◆ Prevented from passing directly into ventricles by barrier of connective tissue, stimulus picked up by atrio-ventricular node
- ◆ Bundle of His composed of Purkyne tissue transmits stimulus to base of ventricles from where it radiates up through the walls back to the connective tissue barrier
- ◆ Detected by electrodes on the skin as an ECG
- ◆ Pacemaker influenced by sympathetic (faster) and parasympathetic (slower) nervous systems, and the hormone adrenaline
- ◆ All influences on heart rate coordinate heart rate and therefore blood supply to body (cardiac output) with demands of body
- ◆ Pressure receptors near entries of great veins detect amount of blood returning to heart in veins, and increase the cardiac output if venous return increases
- ◆ Pressure receptors in the aortic arch and carotid sinus measure pressure of blood being pumped out by the heart, and adjust cardiac output accordingly via the centres in the medulla of the brain.

Response of heart to increased muscular activity

The basic rate of contraction due to the spontaneous rhythm of the sinoatrial node, can be altered by a variety of influences, over a range from about 30 to as many as 220 beats per minute.

The heart is influenced by the activity of the **sympathetic** and **parasympathetic** nerves of the autonomic nervous system, which originate from the cardiovascular control centre in the medulla of the brain.

The sympathetic nerves secrete **noradrenaline**, which stimulates an increase in the heart rate (tachycardia) directly. Also, in response to a general activation of the sympathetic nervous system, **adrenaline** is released from the adrenal glands, which also increases the heart rate.

The parasympathetic nerves, in this case the vagus nerve, releases **acetylcholine** which causes a decrease in the heart rate (bradycardia).

During exercise, the progressive increase in the heart rate is due at first to a decrease in parasympathetic activity, and then to the increasing activity of the sympathetic nervous system. These alterations in the nervous stimulation of the heart are themselves the result of changes associated with exercise acting either directly or indirectly on the cardiovascular control centre in the brain. These changes include the following.

- ▼ An accumulation of carbon dioxide and lactate in the blood.
- ▼ A decrease in the amount of oxygen in the blood.
- ▼ An increase in temperature, which speeds up metabolism in general (and which also reduces the viscosity of the blood making it easier to pump)
- ▼ Nervous reflexes from sensory receptors in the working muscles and joints feeding back information about the increased mechanical activity associated with exercise.
- ▼ An increase in the filling of the heart (as a result of a greater venous return, which in turn occurs as a result of the increased activity of the muscle and respiratory pumps), causes stretching of the SA node, which increases its rhythm of nervous discharge

The heart rate increases very rapidly at the onset of exercise, and in a trained athlete it can treble within a minute of starting exercise. This increase is too rapid to be the result of the activities of the metabolic mechanisms concerned with cardiac acceleration described above, and therefore the exact cause is not clear. However, connections exist between the higher centres of the cerebral cortex and the cardiovascular centre in the medulla of the brain (from where the sympathetic and parasympathetic impulses to the heart originate) which can result in anticipatory increases in heart rate just before exercise commences.

3.3

Energy and exercise

Content:

Energy sources

- ▼ Glucose, glycogen and triglycerides as sources of energy for muscle contraction.
- ▼ ATP as the immediate energy source
- ▼ Comparison of aerobic and anaerobic respiration as sources of ATP for muscle contraction, in terms of energy produced and products.

Muscle fatigue

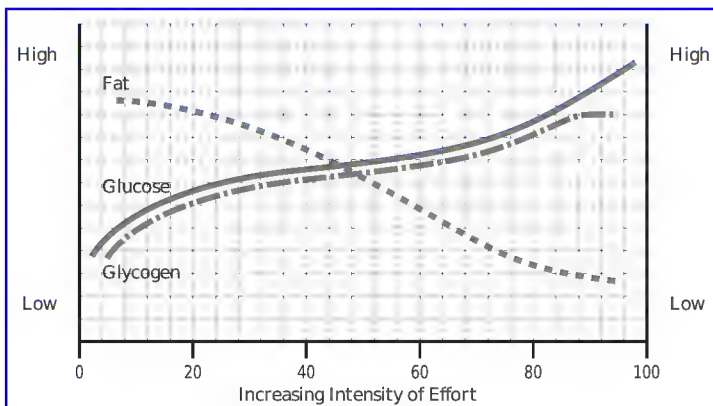
- ▼ Muscle fatigue in terms of increase in blood lactate and decrease in blood pH.
- ▼ The fate of lactate

ENERGY SOURCES

The energy rich materials in the diet, namely carbohydrates, fats, and proteins, are the source of all energy within the body, but the most important sources are glucose, glycogen and triglycerides (fats).

These fuels are interconvertible to a certain extent, depending on their relative availability, and the demands of the body at any one time. The liver is the main centre for these interconversions, and for maintaining constant optimum (homeostatic) levels in the blood. Theoretically most interconversions are reversible, but this is not always true in practice. Thus although the liver can convert excess glucose more easily into fats than into glycogen, very little glucose, if any, can be produced from fats. Also, although liver glycogen is easily converted into blood glucose, muscle glycogen is not.

At rest, about two thirds of the energy required for the body comes from fats, and one third from the carbohydrates glycogen and glucose. The relative amounts of these fuels used in exercise depends on many factors; including the type of exercise (whether it is intermittent or continuous, short or long, light or heavy), the type of muscle fibres involved, the nature of the diet, and the state of physical training - which in turn determines the supply of oxygen.



Glucose and glycogen

Glucose is the end product of carbohydrate digestion. Some of the glucose may be used directly from the blood plasma, and any glucose in excess of the normal levels in the blood is converted either into the insoluble storage carbohydrate glycogen in the muscles and the liver, or into triglycerides which are stored in specialised adipose (fat) tissue under the skin, and around the major organs of the body.

Active muscle fibres can use glucose obtained from all these sources. However, blood glucose is the only source of energy for the brain, and most of the glucose released from the liver glycogen stores is used for this purpose.

Glycogen within the muscle fibres is available for immediate use, for example at the onset of exercise and/or during intense exercise. During these periods, the supply of oxygen is usually insufficient for the complete aerobic oxidation of carbohydrates and fats, and only carbohydrates can be broken down anaerobically to maintain the supply of energy to the contracting muscle fibres.

When oxygen supplies are sufficient, the use of carbohydrates as a fuel has another advantage, that is, for a given volume of oxygen, carbohydrates yield about 12% more energy than fat. The main disadvantage of using glycogen is that stores are very limited when compared with fat, and they can be depleted relatively quickly within a few hours. The depletion of muscle glycogen stores leads rapidly to muscular fatigue. This is why the use of fat is so important the longer exercise continues.

Maintenance of blood glucose levels in prolonged exercise

If the blood glucose levels begin to fall, brain function is affected. Therefore there are safeguards that prevent the muscles from using blood glucose too readily. For example, the ability of the muscles to absorb glucose from the blood is decreased by the reduction in the level of the hormone insulin in the blood (which occurs if the blood glucose falls). Therefore, if blood glucose begins to fall, the muscle fibres are less able to take up plasma glucose and use it as an energy source, but will use glucose from their own glycogen stores, until the glycogen is depleted, at which time they are forced to exploit blood glucose. Eventually, if the use of glucose by the muscles and the brain becomes greater than the supply from the liver, the drop in the blood glucose level (hypoglycaemia), causes fatigue and a proportional decrease in the normal powers of the brain. This is illustrated by the fact that athletes can have great difficulty in making the simplest of calculations in the last few miles of a Marathon.

During exercise, the intake of glucose does not necessarily help. Concentrated solutions remain in the stomach and do not pass easily into the small intestine, where most absorption of water and glucose into the blood normally takes place. Also, if the solution is too concentrated, even if it does reach the small intestine, absorption of water is reduced and water may be drawn out of the blood by osmosis. However, the absorption of water into the blood is increased if the glucose solution is the same concentration as the blood (isotonic), in fact this is the main role of isotonic drinks, rather than energy replacement. During endurance exercise, the problems associated with the intake of energy and water may be overcome by the

use of a solution of long chain carbohydrate molecules (maltodextrins), obtained from the partial breakdown of starch. This provides energy but does not slow the rate of stomach emptying, oppose the uptake of water in the small intestine, or trigger the release of insulin.

Triglycerides

The complete oxidation of fats depends on the presence of oxygen, so the relative contribution of triglycerides to the energy demands of the body is largely determined by the supply of oxygen to the tissues. Fat is the main energy source for muscles under long term aerobic conditions, so after about an hour of continuous aerobic exercise most of the energy is derived from fats. Training increases the capacity for oxygen uptake, and therefore increases the ability to use fats.

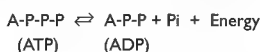
Very little fat is stored in the muscle fibres, therefore the fatty acids and glycerol must be supplied via the bloodstream. The adipose tissue releases free fatty acids (*FFAs*) into the plasma of the blood, which are transported to the muscles loosely bound to plasma proteins. People who take part in regular aerobic endurance work increase their ability to release fatty acids from their fat stores, and can therefore use more fat as an energy source during exercise

The glycerol from the breakdown of fats can either be used directly in respiration, or be converted into glucose which is then broken down in the normal way.

ATP as the immediate energy source

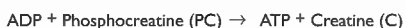
The end product of all the energy releasing processes of cellular respiration is the energy rich compound adenosine triphosphate (ATP). ATP is the universal energy currency of all living things. It is the form in which energy is supplied for all purposes. Each molecule contains three phosphate groups, the end one of which can be more easily displaced than the others, with energy being made available for use by the muscle fibres. In this process, adenosine diphosphate (ADP) and a free inorganic phosphate group (Pi) are formed.

This process is reversible, and energy released from the oxidation of energy rich materials e.g. glucose, can be trapped in the ATP molecule when a phosphate group is combined with ADP to reform ATP.



The relative amounts of ATP and ADP + Pi control the rate of breakdown of energy rich substances in respiration. If ADP and Pi are present, then energy is required to synthesise ATP, and the rate of respiration increases to supply this energy. On the other hand, if only ATP is present, there is no immediate demand for energy, and the rate of respiration decreases.

Only a limited store of ATP occurs within the muscle fibres, and as all energy used by the body must be channelled through ATP, it must be replenished as soon as it is used. To ensure its rapid regeneration there is another energy rich phosphate compound in the muscles, namely phosphocreatine (PC), which acts as the most immediate energy reserve for the resynthesis of ATP. The rapid availability of these is particularly important in fuelling contractions of high power.

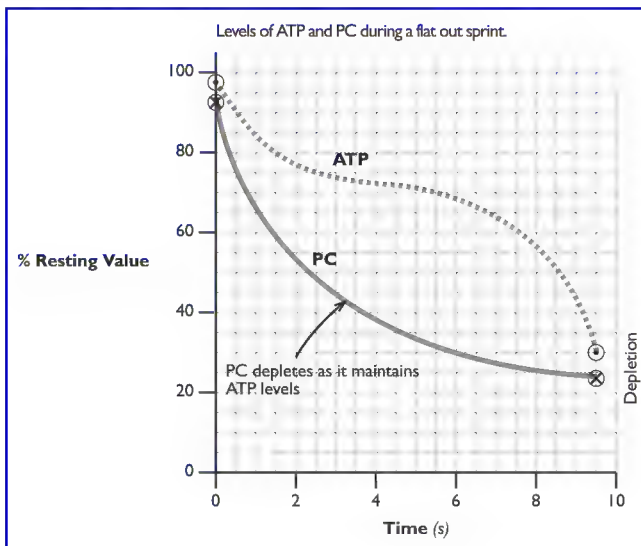


During exercise some ATP can also be regenerated from the combination of ADP molecules:



AMP activates respiratory enzymes, and stimulates the secretion of adrenaline, both of which increase the rate of respiration and therefore the regeneration of ATP from aerobic sources.

It is important to note that all these ways of resynthesising ATP may operate at the same time, but the relative importance of each one depends upon the activity and its duration.



Anaerobic and aerobic respiration

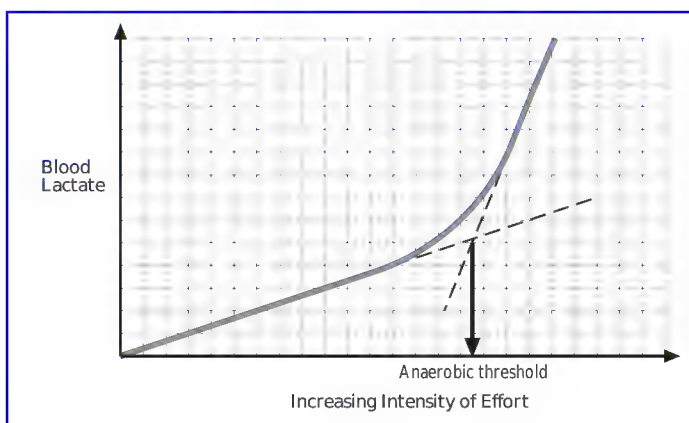
The energy that is trapped and stored in ATP originates from energy rich respiratory substrates e.g. glucose. It is released from the energy rich sources by a series of enzyme catalysed reactions. In these metabolic pathways the respiratory substrates are oxidised to carbon dioxide and water, in the process known as **cellular respiration**. The breakdown of the different respiratory substrates involves common pathways.

The main pathway involves the breakdown of carbohydrates, with the breakdown of fats and proteins linking in at different points.

The initial stages in the breakdown of carbohydrates occur in the cytoplasm of all cells, including muscle fibres. During these initial stages, glucose is broken down to an intermediary product called **pyruvate**. Oxygen is not involved in these initial stages in the cytoplasm and they are therefore referred to as **anaerobic glycolysis**. Anaerobic glycolysis generates a net gain of two ATP molecules from each molecule of glucose, which only represents about 5% of the total ATP that can be generated from the complete breakdown of the glucose molecule. However, this process is important in supplying ATP in high intensity short term exercise as it can supply ATP more than twice as fast as the aerobic system.

When the demand of the muscles for oxygen exceeds the supply, as for example at the beginning of most types of exercise, but particularly during intense efforts lasting up to about a minute, the pyruvate becomes reduced to **lactate (lactic acid)**. The complete breakdown of glucose is thus prevented, as an intermediate product of the breakdown of glucose is being used to form lactic acid, instead of going on to be oxidised to carbon dioxide and water with the release of much larger amounts of energy.

A small amount of lactate is formed even during the lightest exercise, but its rate of removal is equal to its rate of production and it does not accumulate. When the rate of production does exceed its rate of removal, lactate begins to accumulate in the blood. There are normally about 1-2 millimoles of lactate per litre of blood, and it is generally agreed that a level of 4 millimoles per litre of blood represents what has been called the '**anaerobic threshold**'. If the rate of oxygen uptake comes to match the requirement for aerobic respiration during exercise, a 'steady state' exists, and the lactate level in the blood remains relatively constant until the end of the exercise.



If sufficient oxygen is available the pyruvic acid is free to continue down the metabolic pathway that leads to its complete oxidation into carbon dioxide and water, and the production of many more molecules of ATP. The number of molecules of ATP produced from the complete oxidation of one molecule of glucose is disputed. Traditionally the figure quoted is 38, but more recently 31 has been accepted. These aerobic stages take place in the cell structures or organelles known as mitochondria. Muscle fibres have large numbers of mitochondria containing the enzymes, coenzymes, and cytochrome pigments necessary for the aerobic stages. All of these are increased as a result of aerobic training.

Muscle fatigue

Although it is obvious that muscles tire as a result of strenuous use, such are the complexities of muscle physiology that the causes of muscle fatigue are not easily identified. Contributing factors include: interruption of the transmission of nervous impulses, depletion of the sources of energy, alterations in the concentration of the body fluids, increase in body temperature, and insufficient blood supply

An insufficient blood flow to the muscles, by not delivering sufficient oxygen to maintain aerobic respiration, will increase most of these factors contributing to fatigue.

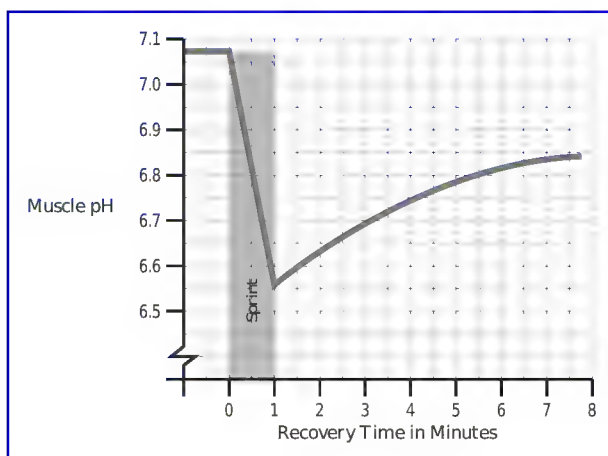
As the oxidation of fat requires more oxygen than the oxidation of glucose, the increasing use of fat during exercise increases the problem of oxygen shortage. With insufficient oxygen, respiration becomes anaerobic and lactate accumulates. The increased acidity (decreased pH) has a complex of effects, all of which contribute to fatigue. It inhibits resynthesis of creatine phosphate, and interferes with muscle contraction. It also inhibits enzymes, especially those involved in glycolysis, and decreases the amount of energy released by the hydrolysis (breakdown) of ATP.

The effect of accumulating lactate is to increase fatigue, and to increase the rate of breathing eventually even past the point at which any extra oxygen uptake can be achieved. It is around the start of this lactate build up during exercise that conversations tend to stop! It is the accumulation of lactate that makes the 400m 'sprint' such a testing event. One effect of training for such high intensity events is to condition the tissues and nervous system to tolerate the high levels of lactate produced during the anaerobic phase.

The fate of lactate

There are several ways by which lactate can be removed from the muscles. Some of them are of relatively minor importance, namely its loss in sweat and urine, and its reversion to glycogen in the liver and in the muscles. This reversion to glycogen in fact requires more energy than was yielded by the original anaerobic glycolysis, so that there is no overall gain of energy if this occurs.

The major pathway for the removal of lactate is its aerobic oxidation to carbon dioxide and water, yielding ATP. This can occur in the muscle fibres themselves once sufficient oxygen is available, especially in the fibres of muscles not taking part in the acute exercise. Or the lactate can be transported in the blood to organs which are not operating under anaerobic conditions, for example the heart, brain, liver, and kidneys, where it can be oxidised. As lactate can be used in this way it is not considered a waste product.



CHECKPOINT SUMMARY

- ◆ The energy rich materials in the diet, namely fats, carbohydrates, and proteins, are the source of all energy within the body
- ◆ The most important direct sources of energy within the body are glucose, glycogen and triglycerides (fats)
- ◆ The end product of all the energy releasing processes of cellular respiration is the energy rich compound adenosine triphosphate or ATP. ATP is the universal energy currency of all living things. It is the form in which energy is supplied for all purposes
- ◆ ATP contains three phosphate groups, the end one of which can be more easily displaced than the others, with energy being made available for use by the muscle fibres. In this process, adenosine diphosphate (ADP) and a free inorganic phosphate group (Pⁱ) are formed
- ◆ This process is reversible, and energy released from the oxidation of energy rich materials can be trapped in the ATP molecule when a phosphate group is combined with ADP to reform ATP
- ◆ Respiratory substrates are oxidised to carbon dioxide and water; in the process known as cellular respiration. The breakdown of the different respiratory substrates involves common pathways
- ◆ The initial stages in the breakdown of carbohydrates do not involve oxygen and they are therefore referred to as anaerobic glycolysis
- ◆ Anaerobic glycolysis generates a net gain of two ATP molecules from each molecule of glucose, which only represents about 5% of the total ATP that can be generated from the complete breakdown of the glucose molecule
- ◆ If sufficient oxygen is available pyruvic acid is completely oxidised into carbon dioxide and water, producing many more molecules of ATP
- ◆ These aerobic stages occur in mitochondria
- ◆ When the demand of the muscles for oxygen exceeds the supply, lactate begins to accumulate in the blood
- ◆ The increased acidity (decreased pH) as a result of the production of lactate has a complex of effects, all of which contribute to fatigue. It inhibits resynthesis of creatine phosphate, and interferes with muscle contraction. It also inhibits enzymes involved in glycolysis, decreasing the amount of energy released by hydrolysis of ATP
- ◆ The major pathway for the removal of lactate is its aerobic oxidation to carbon dioxide and water, yielding ATP mainly in the heart, brain, liver, and kidneys.

3.4

The Transport of Substances in Plants

Content:

Root structure

- ▼ The structure of a primary root: root hairs, endodermis, xylem and phloem. The distribution of these tissues and their adaptations for function

Uptake and the transpiration stream

- ▼ The uptake of water and ions from the soil
- ▼ Pathway of water transport from root hair to stomata including the apoplastic and symplastic pathways in the root.
- ▼ Transpiration and the effects of light, temperature, humidity and air movement.
- ▼ The roles of root pressure and cohesion-tension in moving water through the xylem.

Xerophytes

- ▼ Structural adaptations that reduce the rate of transpiration in xerophytic plants, related to survival in dry conditions.

Translocation

- ▼ Phloem as the tissue that transports organic substances.
- ▼ The mass flow hypothesis for the mechanism of translocation in plants.

Experimental evidence

- ▼ The use of radioactive tracers and ringing experiments to determine the movement of ions and organic substances through plants.

Introduction

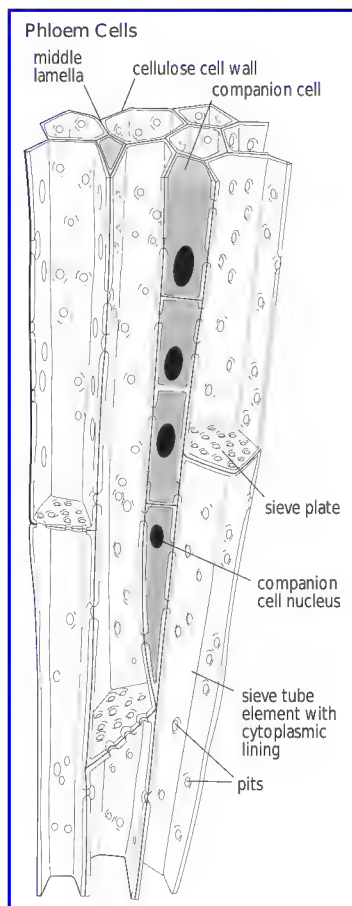
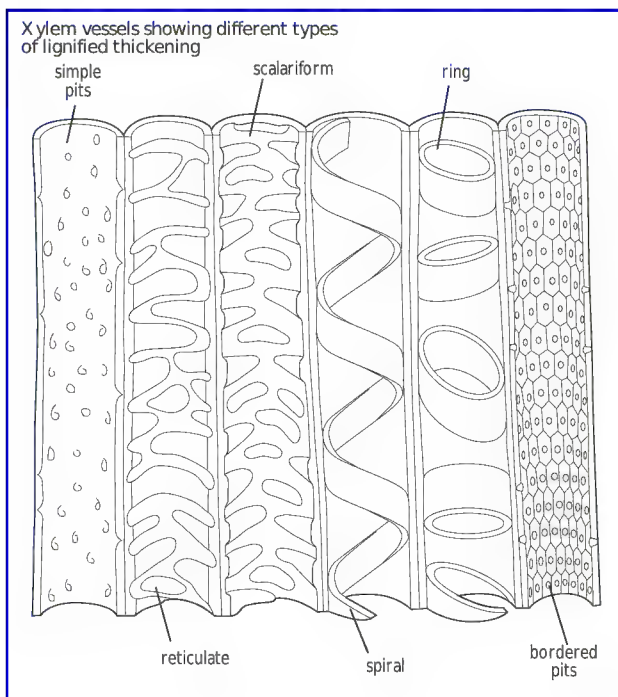
As with animals, simply speaking, the larger the plant, the smaller is its surface area to volume ratio. This relationship is used to explain the development of transport systems to supply internal cells far removed from the exchange surfaces with the environment, and to transport materials between different regions of the plant.

Simple plants, such as the algae, do not have specialised transport systems. Typically they are aquatic or semi-aquatic, and absorb water and nutrients over their whole surface. They also tend to be relatively small, with large surface area to volume ratios which ensures that all parts of the plant are close to the surface and can be supplied by diffusion. Some seaweeds have fronds several metres in length which could not be considered as 'small'. However, the fronds are thin and flattened and have a huge surface area to volume ratio.

Terrestrial plants, namely ferns, conifers and flowering plants, have special problems associated with being rooted in the soil with their aerial parts being exposed to the drying atmosphere. They have true roots, stems and leaves, and can reach a very large size. They have transport systems (vascular tissues) running throughout the plant, the xylem and the phloem. Many of these plants can be relatively small, and become secondarily aquatic (e.g. the water lily, Canadian pondweed etc) but still retain their transport systems albeit in a reduced form. Thus it is important to remember that discussion of the development of transport systems also involves the level of complexity of the organism involved.

Xylem transports water and inorganic ions from the roots up the stems to the leaves for photosynthesis.

Phloem transports sugars (sucrose) mainly from the leaves to the roots and shoot tips for their respiration, or to food storage structures in various parts of the plants.

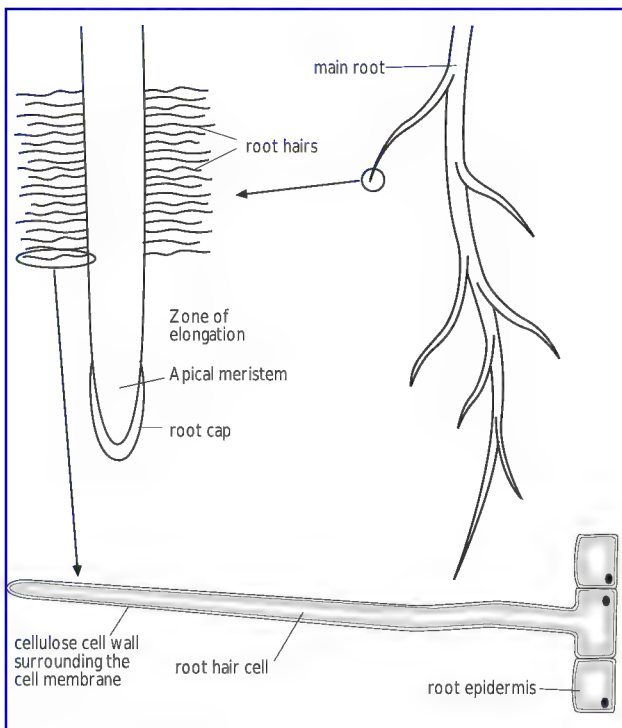
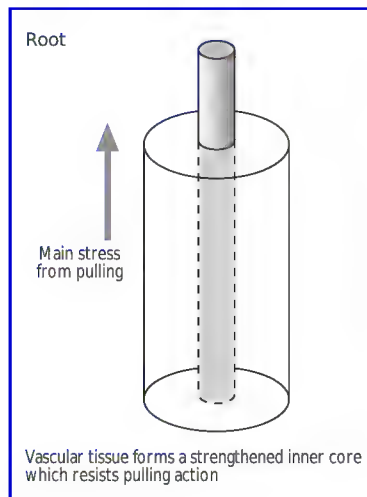
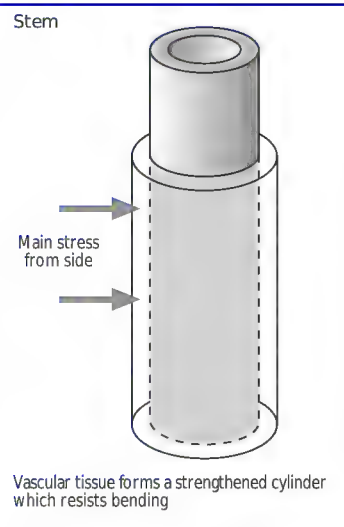


ROOT STRUCTURE

The source of water and inorganic nutrients for plants is the soil. Plant roots are specialised to provide a large surface for the absorption of water and inorganic ions whilst securing the plant in the ground and protecting it from the pulling forces exerted as a result of the action of the wind on the stem and leaves.

The uptake of water and ions occurs only at the root hairs, situated just behind the root tips of newly formed root branches. The hairs are extensions of single, thin walled, epidermal cells. Individually they have a large surface area to volume ratio, and collectively they create a huge surface area for exchanges with the soil.

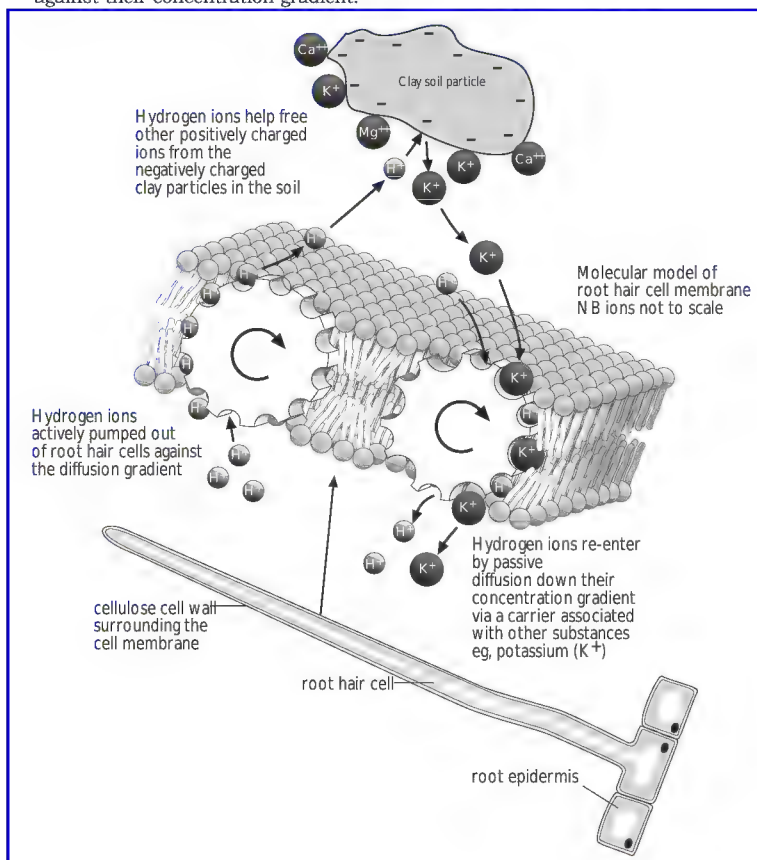
The internal anatomy of roots differs from that of stems in a number of respects, notably the arrangement and position of the vascular tissues (xylem and phloem). As xylem has an important structural and supporting role, its distribution in the root, stems and leaves reflects the mechanical stresses endured by the particular region. In dicotyledonous plant stems, it is arranged in a cylindrical fashion, giving resistance to bending forces created by the wind. In the roots it is concentrated in the centre, resisting pulling forces. (It is interesting to note that aquatic flowering plant species adapted to growing in flowing currents have the stem vascular tissue in the centre, as this best resists the pulling strain of the currents.) In the leaf veins, it ensures that the leaf is supported in a position favourable to photosynthesis.



Another difference between the anatomy of roots and stems is that the root has a cylinder of specialised cells, the **endodermis**, enclosing the central vascular tissues. This had an important role in transport, as you will see later, and crucial to that role is the ring of waterproof thickening (Casparian strip) which seals its cell walls and ensures that all materials transported from the soil solution to the xylem pass through the cytoplasm of the endodermal cells.

THE UPTAKE OF WATER AND THE TRANSPIRATION STREAM

Root hair cells take up inorganic ions from the soil across their membrane by **active transport** (see 10.3). Root hair respiration produces ATP which is used to supply energy for the active uptake of inorganic ions from the soil solution. Respiration also releases hydrogen ions (protons), which are actively pumped out of the root hair cells, setting up a transmembrane potential and creating both the directional stimulus and energy for the inward flow of nutrient ions. The exported hydrogen ions also release other positively charged ions (Na^+ , K^+ , Ca^{++}) from the negatively charged clay particles of the soil and make them more available for active uptake by the roots. By this means ions may be taken up by the plant against their concentration gradient.



If roots are starved of oxygen, as may happen if the soil is flooded, then active mineral uptake will stop, as oxygen is required for the production of ATP in respiration. This has a consequence for water transport because it is the active uptake of mineral ions which provides the gradient along which some water passively enters the root.

As the root hair cells accumulate inorganic ions so a diffusion gradient is created and the absorbed ions tend to move inwards via the cytoplasmic connections (plasmodesmata) between the cells of the epidermis and cortex towards the central vascular region. The endodermal cells assist the passage of ions inwards to the xylem by a further active transport mechanism amplifying the concentration difference between the soil solution and the xylem elements in the centre of the root.

Water entering the root may travel along two pathways.

The **apoplastic** pathway through the porous cellulose walls of the cells of the root cortex, pulled in by the transpiration stream (see below). However, the waterproof Casparian strip gives the endodermis control over this process, effectively blocking the apoplastic route and ensuring that all water passes through the cytoplasm of the living passage cells of the endodermis.

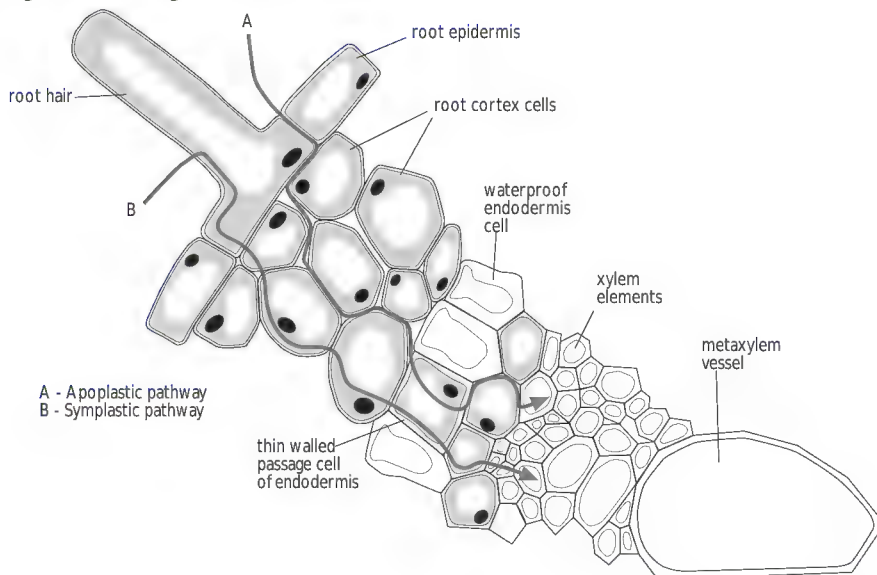
The **symplastic** pathway through the cell membranes and cytoplasm of the cells of the cortex of the root down a water potential gradient by osmosis.

The apoplastic pathway offers least resistance, as the water does not have to pass through any living membranes or cytoplasm (except at the endodermis), and most water passes across the root this way. The symplastic pathway is a route of high resistance as the water must pass through the cell membranes (and possibly vacuolar membranes) and cytoplasm, and is mainly involved in the supply of water to individual root cortex cells from the main flow of water streaming across into the xylem.

● CHECKPOINT SUMMARY

- Roots provide anchorage, and take up water and inorganic ions from the soil solution.
- As roots have to resist predominately pulling strains, the vascular (transport) tissue containing the phloem and woody xylem is found in the centre.
- Uptake of water and ions from the soil solution is via the root hairs which present a large total surface area for uptake.
- Root hairs are found just behind the actively growing root tips of young roots.
- The endodermis is a cylinder surrounding the central vascular tissue, it is composed of cells the walls of which become impregnated with impermeable waterproof suberin and eventually die, except for living passage cells next to the points of the central 'star' of xylem tissue.
- The endodermis acts as a barrier to prevent the loss of accumulated ions in the root, and to force the water and ions being taken up to pass through the contents of living cells at least once in its passage across the root.
- The xylem tissue transports water and ions up to the stem and leaves, and the phloem transports organic substances especially sucrose from the leaves to the roots.

Passage of water through the root



Transpiration

Transpiration is the loss of water from the leaves by evaporation. Although a tiny amount of water may be lost directly through the 'waterproof' cuticle of the upper and lower epidermis of the leaves, virtually all of it occurs through the stomata of the leaves when they are open. Transpiration is an unavoidable consequence of gaseous exchange. As stomata open in the light to take in carbon dioxide for photosynthesis, so water vapour escapes. However, transpiration does have some beneficial effects, it provides a transport stream (transpiration stream) bringing water and mineral ions to the leaves from the roots. It also exerts a cooling effect. Under very hot conditions, however, when the cooling effect is most required, the stomata tend to close, thus stopping transpiration.

Factors affecting transpiration include anything that affects the supply of water to the leaves, and the evaporation of water from the leaves. The main factors are:

Availability of soil water determines the supply of water to the plant. Any factor that decreases the availability of soil water will decrease transpiration, which eventually can lead to the stomata closing and in extreme cases to wilting of the plant.

Relative humidity is a measure of the water vapour content of the air. The diffusion of water vapour out through the stomata of a leaf only occurs when the relative humidity of the surrounding air is lower than that of the internal leaf spaces. The rate of transpiration increases with decreasing relative air humidity. Relative humidity is dependent mainly on temperature, decreasing as temperature increases. A rise of 10°C doubles the steepness of the humidity gradients from leaf to air, thus increasing transpiration.

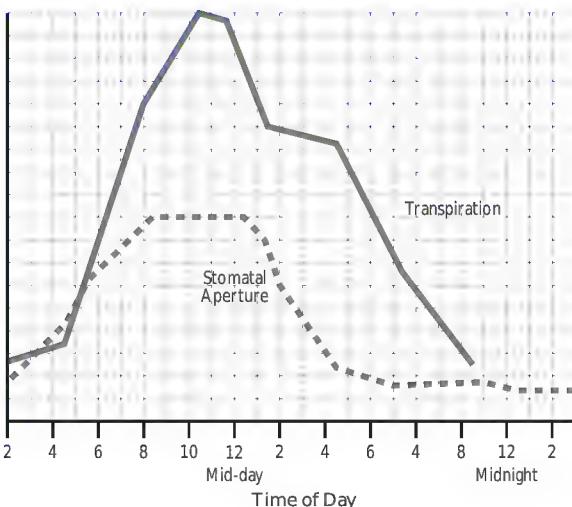
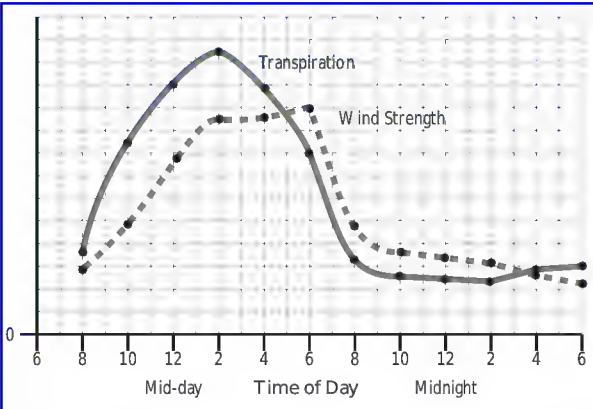
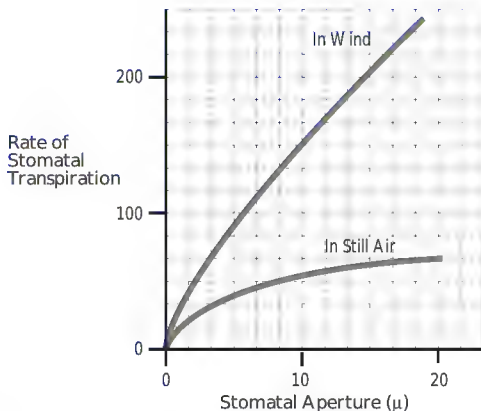
Air movements increase the rate of water loss by transpiration as molecules of water vapour are carried away from the leaf surface reducing the relative humidity of the air that is immediately surrounding it.

Temperature increase provides energy for an increase in evaporation of water from the leaf.

Light intensity exerts its main effect directly on the guard cells surrounding the stomata. These are the only cells on the surface of the leaf which possess chlorophyll, and an increase in light intensity typically results in their daytime opening and a consequent increased transpiration rate. Indirectly it will also raise the temperature of the leaf as its radiant energy is absorbed.

Stomatal number and size vary widely between species, typically being directly correlated with the availability of water. There are usually more stomata on the lower side of leaves. Some plants (e.g. laurel) have stomata only on the lower side. Grasses having vertical leaves have roughly equal numbers on both surfaces. On average there are about 300 per mm² of leaf surface.

Stomata allow the uptake of carbon dioxide for photosynthesis but at the same time they control the rate of water loss. Changes in stomatal size affect water loss more critically than carbon dioxide gain, i.e. if the pore size is reduced, the transpiration rate is reduced more than the rate of carbon dioxide uptake.

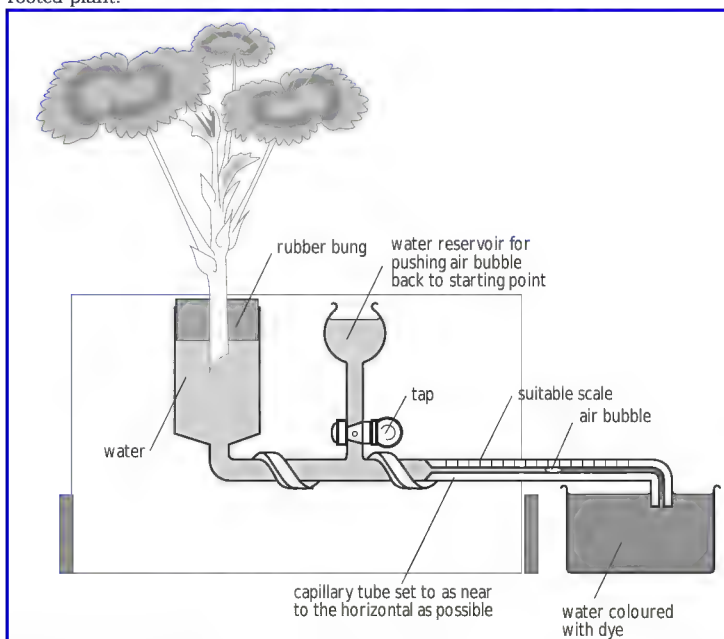


◆ CHECKPOINT SUMMARY

- ◆ The uptake of water from the soil is by osmosis when it is actually entering and passing through living cells (symplast pathway), and cohesive pull of the transpiration stream when it is passing through the porous cellulose cell walls directly to the xylem (apoplast pathway).
- ◆ The uptake of ions is by diffusion, facilitated diffusion and active uptake, and they may be swept up by solvent drag via the apoplast pathway.
- ◆ In all cases the passage cells of the endodermis exert some control.
- ◆ The active accumulation of ions within the barrier formed by the endodermis followed by the passive uptake of water by osmosis, results in the development of a root pressure which can force water up the xylem for a limited distance.
- ◆ Transpiration is the loss of water vapour from the leaves.
- ◆ It is an unavoidable consequence of having stomata opening for the uptake of carbon dioxide (and release of oxygen) in the light.
- ◆ Evaporation provides upward movement of a water column held together by cohesion (cohesion-tension) between water molecules in the xylem - the transpiration stream.
- ◆ The transpiration stream provides for upward transport of water and dissolved inorganic ions (and some organic compounds) from roots to leaves.
- ◆ Evaporation from leaves provides some cooling effect, but not when most needed as stomata shut and leaves wilt in conditions of extreme heat.
- ◆ Transpiration rate is affected by any factor that affects evaporation of water: relative humidity, temperature, air speed, availability of water, light intensity, degree of opening of stomata, number and distribution of stomata.
- ◆ Anything that increases the water potential gradient between the interior of the leaf and the external air increases transpiration.

Experimental investigation of factors affecting transpiration rate

Investigations of the effect of many of these factors can be carried out using a piece of laboratory equipment, the potometer, in which a cut shoot is exposed to different conditions and its water uptake is measured. The rate of water uptake is assumed to be the same as the rate of transpiration, but with a cut shoot the cross sectional area of the cut stem is not as great as the surface area of roots that would normally supply water to that shoot. The transpiration rate from the shoot would therefore be greater if still attached to the rooted plant.



◆ CHECKPOINT SUMMARY

- ◆ Transpiration can be measured using a potometer. The principle of which, is that as the water evaporates from the leaves of the shoot, water is moved up the shoot by the cohesive pull of the transpiration stream, and an air (bubble) is drawn along the capillary tube at the same rate - thus giving a measure of the rate of transpiration. The time taken for the air bubble to travel a certain distance, or the distance travelled in a certain time, can be recorded and the rate of transpiration calculated.
- ◆ Actually it is the rate of water uptake that is being measured, which under these experimental conditions is not the same as the transpiration rate. In this experiment the cross sectional area of the cut shoot is not as large as the equivalent area of root hairs that would normally supply the leaves, therefore the rate of uptake is less than the rate of transpiration.
- ◆ The potometer is filled by immersing it totally in water; preferably freshly boiled and cooled water which has no air bubbles.
- ◆ The shoot to be used should be cut from the plant and fitted into the potometer under water; to prevent any air bubbles entering the xylem elements.
- ◆ All joints should be well vaselined to seal leaks
- ◆ If the calibration on the capillary tube is in millimetres, and the diameter and hence the radius of the tube is known, then the volume of water taken up can be calculated using the formula: $\pi r^2 \times \text{distance travelled by bubble} = \text{volume of water uptake}$
- ◆ Sometimes the calibrations are already in units of volume, for example mm^3 or cm^3 (ml). After the bubble has travelled a certain distance, it is returned to the beaker end by running water in from the reservoir.

Pathway and mechanism of water transport from roots to leaves

Transpiration provides the main force for water transport from root to leaf. The greater bulk of water is pulled in directly by the transpiration stream, acting through a continuous system of water between the soil solution and the xylem in the porous cellulose walls of the cells, the apoplastic pathway.

Water and dissolved solutes move up the stem in the xylem tissue. The generally accepted explanation of the mechanism for the upward transport of water is known as the **cohesion-tension mechanism**. It is based on purely physical forces and does not involve the activities of living cells. Evaporation through the leaf pores or stomata causes a gradient of water potential across the mesophyll cells of the leaf, and a direct force on the water in the porous cellulose cell walls which causes water to be withdrawn from the xylem. Cohesive forces between water molecules result in a column of water being drawn up the plant as the water evaporates from the leaves. This transpiration 'pull' generates a tension which results in the sap being under a negative pressure. This can produce a measurable decrease in stem diameter (and even trunk diameter) when transpiration is high. The water column is supported and prevented from falling back down under gravity in the xylem elements by adhesion to the lignified walls of the xylem elements. A continuous water column is necessary for this mechanism, and any breakages caused by storm damage or freezing (which forces air out of solution) can be by-passed via the system of lateral pits in the lignified walls. In woody plants only the most recently formed 'sap wood' functions in water transport, and the remaining 'heartwood' is used as a depository for waste products (but is still important in support).

This mass flow of water carries along dissolved inorganic ions in solution by 'solvent drag' and contributes to the uptake of inorganic ions from the soil solution by the plant.

Root pressure

At night, the transpiration stream slows down or stops altogether but root hair cells continue the active uptake of ions. The xylem contents therefore become relatively more concentrated at night and, as their water potential is lowered, so water moves by osmosis out of the surrounding cells of the root cortex. This creates a positive pressure of fluid within the root xylem called **root pressure**, the effects of which can be observed by cutting the stem of a plant just above ground and measuring the force with which fluid flows out of the cut. Root pressure is not a mechanism of water transport; rather a by-product of the active uptake of ions by roots. It is very much less evident during the day when the plant is transpiring because the accumulating ions in the root xylem are quickly carried out of the root by the transpiration stream.

◆ CHECKPOINT SUMMARY

- ◆ Thickened waterproof cuticle reduces water loss through the surface of the leaf, especially on the upper surface of the leaf which is most exposed to air currents and heat absorption from sunlight
- ◆ Surface of the leaf may be 'hairy' (not true hairs) which protect the stomata from air currents
- ◆ Stomata can be sunken in pits and grooves, which protects them from air currents, e.g. on pine 'needles'
- ◆ Stomata can be reduced in number, especially on the upper surface of the leaves, where the exposure to the heat of the sun and air currents is the greatest, indeed some e.g. laurel, have no stomata on the upper surface
- ◆ Leaves can have a reduced surface area, which also reduces the number of stomata, e.g. pine needles; and gorse and cacti where the leaves are reduced to spines
- ◆ Leaf folding or rolling reduces water loss through the stomata by protecting them from air currents, and enclosing them in a zone of high humidity, e.g. *Ammophila* (sand dune grass)
- ◆ Leaves can be succulent, with tissues where water can be stored for periods of drought, e.g. *Mesembryanthemum*
- ◆ Cells of the leaves have mucilage and can withstand dehydration for longer periods than those of non-xerophytes (mesophytes)
- ◆ Leaf fall stops transpiration. Deciduous plants lose their leaves with the onset of winter in temperate regions, when the low temperatures inhibit water uptake by the roots.

XEROPHYTES

Plants adapted to growing in conditions of water shortage are known as xerophytic plants or xerophytes. This group includes plants from desert regions, as well as those in temperate regions on rapidly draining sandy soils, or in salt marshes.

As has been seen the main way in which terrestrial plants lose water is by evaporation from their leaves, a process known as transpiration. Some water is lost in this way over the whole leaf surface through the cuticle (cuticular transpiration), but most is lost through the stomata, which typically open in the light for the absorption of carbon dioxide for photosynthesis. Xerophytes show many structural adaptations of the leaves which decrease the rate of transpiration, and individual species can show any combination of these.

A thickened waterproof cuticle reduces water loss through the surface of the leaf, especially on the upper surface which is most exposed to air currents and heat absorption from sunlight, both of which increase the rate of evaporation of water from the leaf in transpiration; e.g. holly leaves.

The surface of the leaf may be 'hairy' (not true hairs) which protect the stomata from air currents which would otherwise increase transpiration e.g. 'Viper's Bugloss' (*Echium vulgare*).

Stomata can be sunken in pits and grooves, which protect them from air currents, e.g. on pine 'needles'.

Stomata can be reduced in number, especially on the upper surface of the leaves, where the exposure to the heat of the sun and air currents is the greatest, indeed some e.g. laurel, have no stomata on the upper surface.

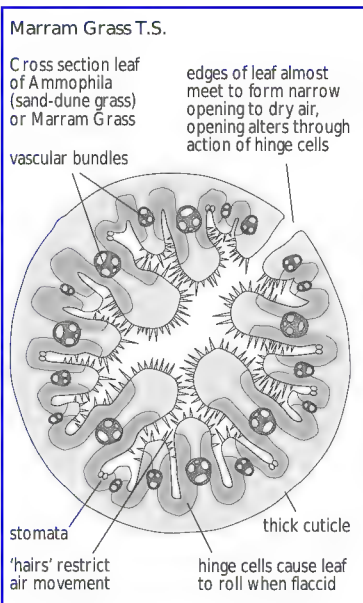
Leaves can have a reduced surface area, which also reduces the number of stomata, e.g. pine 'needles'; and gorse and cacti where the leaves are reduced to spines and photosynthesis is carried out by the green stems.

Leaf folding or rolling reduces water loss through the stomata by protecting them from air currents, and enclosing them in a zone of high humidity, e.g. sand dune grass (*Ammophila*), where the long ridged leaf rolls up along its length at times of excessive transpiration.

Leaves can be succulent, with tissues where water can be stored for periods of drought, e.g. cacti.

Cells of the leaves have mucilage and can withstand dehydration for longer periods than those of non-xerophytes (mesophytes), e.g. cacti.

Leaf fall stops transpiration. Deciduous plants lose their leaves with the onset of winter in temperate regions, when the low temperatures inhibit water uptake by the roots, (you may have noticed that some of the xerophytes mentioned above (pine and laurel) are 'evergreens', i.e. they do not lose their leaves in winter as they have a low transpiration rate). Some plants lose their leaves in dry periods for the same reason, and photosynthesis is carried out by the green stems e.g. broom.



TRANSLOCATION

Translocation is the name given to the energy-requiring process in which organic materials synthesised by the plants, particularly sucrose are transport in the phloem sieve tube elements.

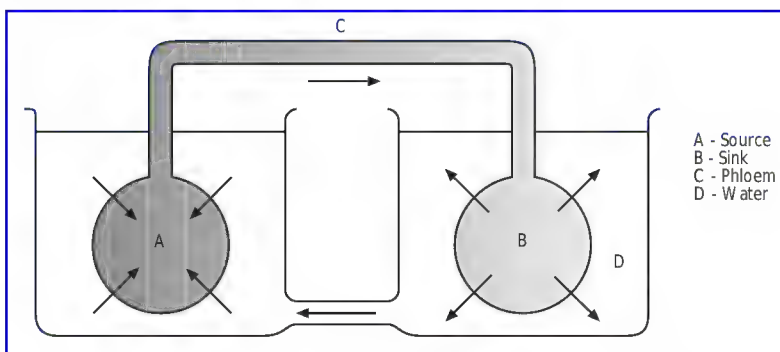
Organic substances synthesised by plants are also known as **assimilates**, as they have been synthesised (assimilated) from simple inorganic substances.

Translocation from sources to sinks

The transport of sucrose and other organic compounds occurs from the point of origin or 'source' of the organic material to the point of destination or 'sink'. Typically the main 'source' of sucrose is the photosynthesising leaves. **Transfer cells** found next to sieve tubes, especially in small leaf veins, have a characteristic dense cytoplasm and many organelles, and appear to be involved in transferring substances to and from the sieve tubes of the phloem. The main 'sink' is the respiring roots. However, other 'sinks' are shoot tips, fruits and seeds, and food storage organs e.g. tubers and bulbs, in the autumn. Tubers and bulbs can in turn act as 'sources' and the growing shoots and leaves as 'sinks' in the spring. Thus, transport of sucrose and other organic compounds in the phloem can be in two directions, unlike the xylem where transport of water is only in one direction from the roots towards the leaves. If the 'source' is in the roots and the 'sink' is in the aerial parts, then some organic material can move in the transpiration stream up the xylem.

Mechanism of transport in phloem

Diffusion is not sufficient to account for the observed rates of flow in the phloem. The pressure flow (**mass flow**) mechanism suggests that the 'source' regions have a lower water potential than the 'sinks', due to the presence of greater concentrations of solutes, such as sucrose. This results in water uptake and an increased turgor within the cells at the source. As a result of this and the fact that the two regions are in direct cytoplasmic contact, it is suggested that the organic materials are forced along the resultant hydrostatic (water) pressure gradient, for example from leaves to roots. The flow being maintained by active pumping of sugars into the sieve tube elements in the leaves by companion-transfer cells, and their removal at the 'sink' as a result of their use (e.g. in respiration or conversion to insoluble starch).



EXPERIMENTAL EVIDENCE

Aphids (greenfly) feed specifically on living phloem by means of their long tubular mouthparts which they can insert directly into the phloem sieve tube elements. If they are knocked off whilst feeding, their mouthparts are left protruding from the phloem, and the sugary contents, referred to as 'honey dew' exude out of the broken ends for periods as long as several days, in volumes up to 100 000 times the volume of the one penetrated sieve tube element. This demonstrates that the contents of the sieve tube elements are indeed under positive pressure as the mechanism would require (in contrast to the negative pressure of the contents of the xylem elements), and that the flow is significant. On a larger and less precise scale, cut phloem in large woody plants can exude sucrose rich sap in volumes up to many litres (dm³) per day.

Direct measurements of the pressure within the sieve tube elements indicate that the pressure gradients between sources and sinks are in the right direction and great enough to account for the mass flow of sugars over the largest distances required in the tallest trees.

Evidence against this mechanism

Evidence against this mechanism is provided by the fact that the movement is not simply from source to sink. Mature leaves kept in the dark, unable to photosynthesise and supply their own sugars for respiration, do not import sugars and eventually die. This observation supports the suggestion that the growth substance auxin is in some way involved. Mature leaves have low auxin levels, whereas virtually all 'sinks' are actively growing tissues e.g. roots, food storage organs and shoot tips, and have high auxin levels. Furthermore, when auxins are applied artificially to a region of a plant the flow of sucrose in the phloem is redirected towards it.

Observations that translocation relies on the activities of living cells, argue against the simple mass flow mechanism, which could operate more easily through dead empty elements like the xylem, as long as the loading and unloading regions were living. These observations include the following:

- ▼ translocation only occurs in young phloem sieve tube elements with actively streaming cytoplasm;
- ▼ metabolic poisons (e.g. those inhibiting respiration) inhibit translocation;
- ▼ when the phloem tissue is killed with heat or poisonous substances translocation stops;
- ▼ companion cells are metabolically active along the length of the sieve tube elements, suggesting some role in translocation.

The use of radioactive tracers and ringing experiments to determine the movement of ions and organic substances through plants

Certain substances (elements) have radioactive forms (radioactive isotopes). Radioactive isotopes have the same chemical properties as the non-radioactive form of the element, but are unstable and emit radiations known as 'radioactivity'. These emissions can be detected by a Geiger counter, and by photographic film to produce an

autoradiograph, so that the presence of the radioactive isotope of an element can be located. As radioactive isotopes have the same chemical properties as the normal form, they can be used as **radioactive tracers** to trace the path of an element such as carbon (and compounds containing that element such as sucrose) through the plant.

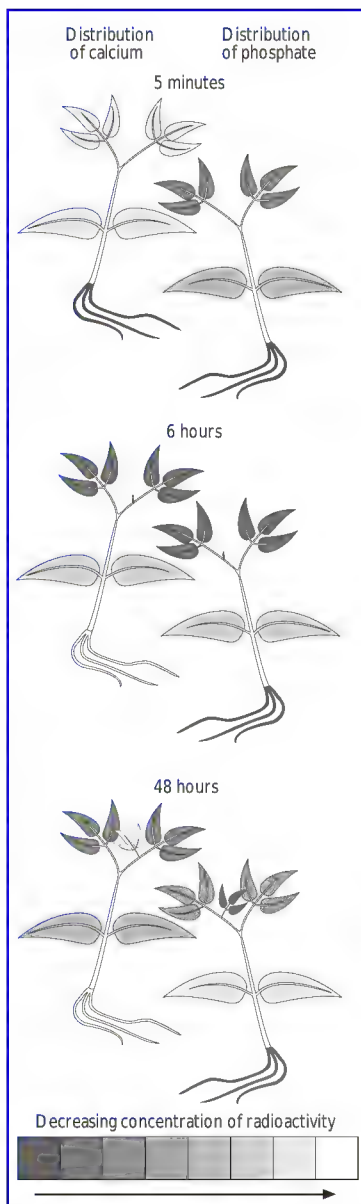
The roots and leaves of plants can be exposed to radioactive isotopes and their subsequent movement through the plant can be traced to determine the movement of ions and organic substances through plants in the xylem and phloem. As the phloem is situated outside of the xylem in woody stems, it can be removed easily by '**ringing**' leaving the central xylem intact, allowing insights into the roles of the xylem and phloem.

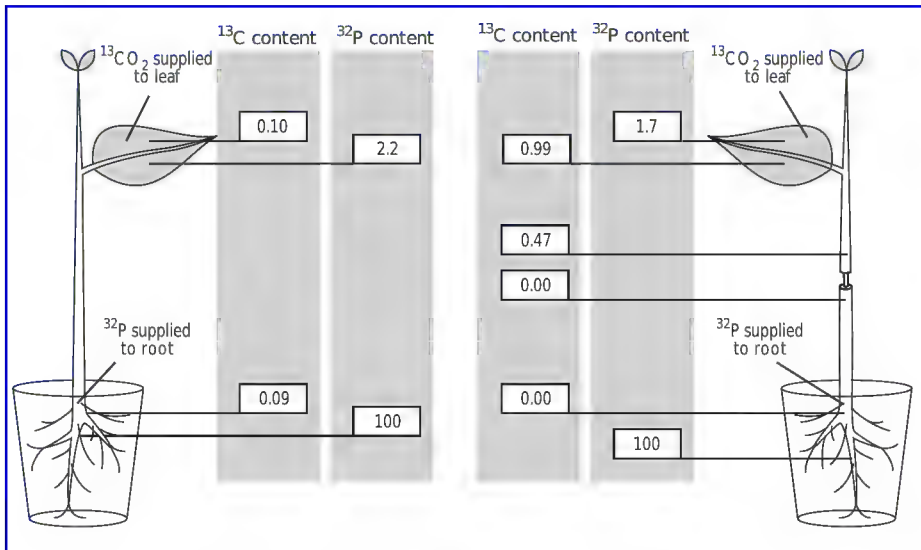
If the roots are exposed to radioactive calcium and phosphorus in solution for an hour, and then removed to a non-radioactive solution, and pressed onto photographic film after 5 minutes, 6 hours, and 48 hours, the uptake and subsequent movement of the ions in the plant can be studied. It can be seen that the phosphate ions quickly reach the young leaves, and transfer to each batch of new young leaves as they emerge, whereas the calcium arrives more slowly in the young leaves and remains there. Ringing the stem has no effect on these movements, indicating that the movement up the plant is in the xylem. However, for the phosphate ions to move from one set of leaves to another, they must also be moved in the phloem. The different rates of arrival in the leaves indicate that their uptake and movement in the xylem differ, not what would be expected from them being taken up by solvent drag and being swept passively along in the transpiration stream.

When leaves are exposed to radioactive carbon in carbon dioxide, the radioactivity eventually reaches the roots, but ringing the stem (removing the phloem) stops this downward movement, indicating that it occurs in the phloem.

Sections of the stem can be taken and autoradiographs produced, but lateral transfer occurs between xylem and phloem so the results can be inconclusive, for example when radioactive ions are taken up by the roots they appear in both the xylem and the phloem. Waxed paper barriers placed between the xylem and the phloem prevent this and the radioactive ions appear mainly in the xylem.

Radioactive tracer studies also indicate that substances move in different directions at the same time in the phloem, which would argue against the mass flow mechanism, although this could be occurring in different sieve tube elements, which would still be explicable in terms of the mass flow theory.





◆ CHECKPOINT SUMMARY

- ◆ Translocation of assimilates (organic substances synthesised in the plant by photosynthesis) e.g. sugars, amino acids, hormones, nucleic acids etc. occurs in the phloem tissue
- ◆ Organic materials move from an area of manufacture (source) to an area where they are used (sink). Sucrose thus moves from the leaves (sources) to actively growing and respiring regions such as the roots, flowers, and new buds (sinks)
- ◆ Phloem tissue (unlike most of the Xylem) is composed of living cells and elements
- ◆ Mechanism of movement still not fully understood
- ◆ Mass flow mechanism postulates high hydrostatic pressure in sources and low in sinks, which results in the mass flow of sucrose in solution from sources to sinks
- ◆ Contents are under positive pressure but there is no clear evidence of the necessary pressure gradients
- ◆ Movement occurs in both directions, although this could be accounted for by considering the mass flow mechanism in respect to single columns of sieve tube elements within the phloem tissue as a whole
- ◆ Movement of assimilates is stopped if the phloem is poisoned with a respiratory inhibitor; exposed to high or low temperatures i.e. translocation is an active process, only occurring in those sieve tube elements showing cytoplasmic streaming
- ◆ Radioactive tracers and ringing experiments are used to determine the movement of ions and organic substances through plants
- ◆ Ringing removes all tissues (including the phloem) outside of the xylem, and radioactive tracers e.g. ^{32}P and ^{14}C allow the movement of materials in the xylem and phloem to be detected
- ◆ ^{32}P supplied to the roots will pass through the ringed region, whereas ^{14}C taken up the leaves and assimilated into sucrose will accumulate above the ring.

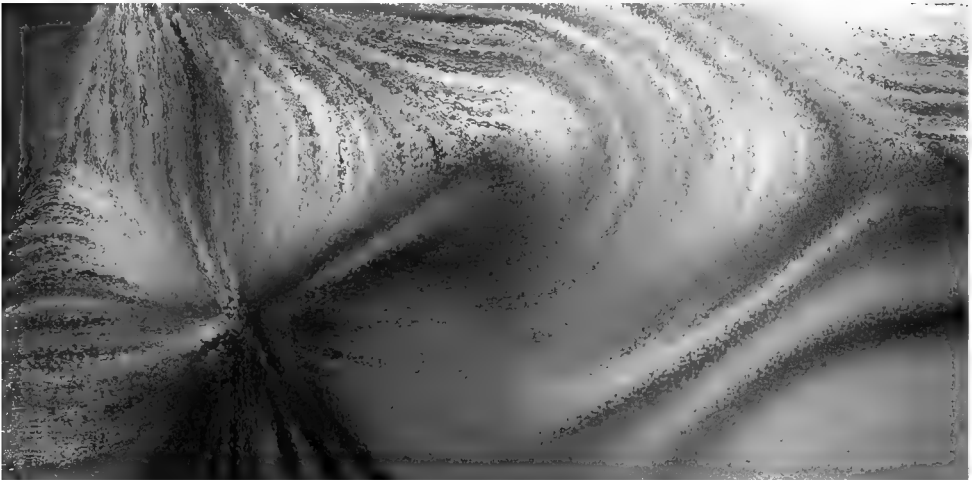
Biology



- 1 - Core Principles**
- 2 - Genes and Genetic Engineering**
- 3 - Physiology and Transport**

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Topic Guide



Topic Guide



1.1 Biological Molecules

1.2 Cells

1.3 Cell Transport

1.4 Exchange of Materials with the Environment

1.5 Enzymes

1.6 Digestion

Core Principles

1.1 Biological molecules Carbohydrates

Topic Guide

Session:

Check List

- ▼ Simple formula for glucose - $C_6H_{12}O_6$
- ▼ Forms straight chain in dry powder form.
- ▼ Forms ring structure in solution.
- ▼ alpha and beta glucose are isomers differing in the 3D arrangement of their atoms.
- ▼ Monosaccharides (glucose, fructose and galactose) combine together with the elimination of water to form glycosidic bonds - a condensation reaction.
- ▼ Glycosidic bonds are broken by the addition of the elements of water across the bond (hydrolysis reaction).
- ▼ Starch is a mixture of amylose (unbranched chains) and amylopectin (branched chains).
- ▼ Both being long chains (polymers) of alpha glucose molecules.
- ▼ When combined the alpha glucose molecules have their oxygen bridges on the same side of the chain, hydrogen bonds form between them and cause the chain to coil into a helix.
- ▼ Starch molecules are relatively insoluble in water.
- ▼ Cellulose is a polymer of beta glucose which have their oxygen bridges on alternate sides of the chain and so cannot form a helix.
- ▼ Instead they form hydrogen bonds with other adjacent chains to make flat ribbons.

Student Activity

Materials

- Molecular model of alpha and beta glucose.
- Small tasting samples of glucose, fructose, sucrose in powdered form on sterile filter papers
- ☐ OHT Monosaccharides and disaccharides
- ☐ OHT Polysaccharides

Procedure

- ☐ OHT Monosaccharides and disaccharides recaps structure of alpha and beta glucose

Observe molecular models of glucose.

- ☐ OHT Polysaccharides

'Blind' tasting of fructose, glucose and sucrose.

Put the substances in order of sweetness. Discuss how properties can vary with small molecular differences. (Caution - do not use galactose. Some people are intolerant).

Set work

Make molecular diagrams to show the structure of alpha and beta glucose, and maltose.

Notes:

Core Principles

1.1 Biological molecules

Proteins

Topic Guide

Session:

Check List

- ▼ All amino acids share a common structure consisting of a hydrogen atom, an amino group (NH_2), a carboxyl acid group (COOH), and an 'R' group, all attached to a carbon atom.
- ▼ The 'R' group can range from a single hydrogen atom to a long complex chain. Thus the simplest amino acid has the formula of $\text{CH}_2\text{NH}_2\text{COOH}$.
- ▼ Amino acids combine in a condensation reactions which form a peptide bond.
- ▼ There are about 20 different types of amino acids found in proteins of living organisms.
- ▼ Proteins are polymers of up to 2000 amino acids joined by peptide bonds (polypeptides)
- ▼ The combination of the 20 different types of amino acids gives rise to as many as 100 000 different types of proteins in living organisms.
- ▼ The sequence of amino acids in the polypeptide chain determines the primary structure of proteins.
- ▼ Hydrogen bonds between adjacent NH_2 and COOH groups result in the secondary structure of either alpha helix or beta sheet. Both of these structures may occur in the same protein.
- ▼ Bonds that form between the 'R' groups determine the tertiary structure.
- ▼ Combination or association of two or more polypeptide chains determines the quaternary structure of a protein.
- ▼ Haemoglobin has a quaternary structure involving four polypeptide chains and four iron containing haem groups.

Student Activity

Materials

- Molecular models of two amino acids, one with an 'R' group of just one hydrogen atom, and one with a long chain.
- Popper beads.
- ☐ OHT Amino acid structure and peptide bond
- ☐ OHT Secondary and tertiary structures of proteins
- ☐ OHT Structure of haemoglobin

Procedure

- ☐ OHT Amino acid structure and peptide bond

Observe 3D models of amino acids.

Form a peptide bond between them.

- ☐ OHT Secondary and tertiary structures of proteins

Explain the different R group bonds: hydrogen, ionic, disulphide and hydrophobic (see text)

OHT haemoglobin molecule and allow students time to make notes.

Set work

In what ways are the structure of Aspartame, insulin, collagen and haemoglobin similar.

In what ways are they different.

Explain why egg albumen changes from liquid to solid (egg white) when cooked.

Notes:

Core Principles

1.1 Biological molecules

Biochemical tests

Topic Guide

Session:

Check List

- ▼ Generally all these tests are qualitative, i.e. the results show whether a substance is present or not, but they do not show how much
- ▼ They are not generally quantitative, i.e. the results do not give an accurate measure of how much of the substance is present.
- ▼ The Benedict's test for reducing sugars can be semi-quantitative in so much as the colour and amount of the precipitate found in a positive test is roughly proportional to the amount of reducing sugar present. Positive results can be compared to results from solutions of known concentration of reducing sugar.
- ▼ The same considerations apply to the iodine in potassium iodide test for starch. The colour for a positive test grading from blue-black to pale brown with decreasing amounts of starch present.
- ▼ The test for lipids is physical rather than chemical, depending on the observation of the formation of an emulsion.
- ▼ The biuret test is not a simple test for proteins but is used as such by Biologists
- ▼ These tests are commonly referred to as 'Food' tests as traditionally they are used on samples of food.

Student Activity

Materials

- Practical Work Sheet:
Simple biochemical tests
- Safety equipment

Procedure

Follow the detailed instructions on the 'Practical Work Sheet.

Set work

Write up and tabulate the results of all your tests.

Comment on any observations that you recorded that are outside of the normally expected results.

Notes:

Session:

Chromatography

- ▼ The separation may be enhanced by performing a second run of solvent, this time at right angles to the first, producing a two way chromatogram.

Notes:

If a chromatogram has been obtained, sellotape it into your practical folder and annotate it with regard to the separation that has been achieved.

Core Principles

1.1 Biological molecules

Water

Check List

- ▼ Water molecules are dipolar with an electronegative pole and an electropositive pole.
- ▼ The dipolar nature of water causes molecules to be attracted to each other by hydrogen bonds resulting in most of the key properties of water in relation to living organisms.
- ▼ Liquid at normal temperature and pressures, solid (ice) at 0°C, boiling point 100°C, providing a wide range as a liquid.
- ▼ It has high cohesion resulting in a strong surface film allowing small organisms to be supported on top or underneath it, and the ability for the water column in the xylem not to break under tension.
- ▼ 'Universal' solvent in which many substances dissolve.
- ▼ Water supports aquatic organisms, ice is less dense than water, therefore ice floats and organisms can survive beneath it.
- ▼ Low viscosity allows free flow for transport of materials inside and outside of organisms.
- ▼ Water rises in small bore tubes by capillarity e.g. important in water transport in plants.
- ▼ Being incompressible water provides a hydrostatic skeleton, e.g. earthworm body cavity, human abdominal cavity
- ▼ Latent heat of evaporation results in a cooling process e.g. sweating
- ▼ Water has a high specific heat which is important in temperature control of aquatic environments and internal fluids.
- ▼ Transparency allows the transmission of light e.g. for photosynthesis

Student Activity

Materials

- 2 thermometers with small piece of blotting paper and 2 rubber bands
- Slices of cucumber (5mm wide)
- Small squares of cellophane (2-3cm)
- Pieces of capillary tubing (6-10 cm), and tubing of wider diameter
- Collection of 5p coins

Procedure

Put cucumber slice into water and see how many 5p coins it take to sink it.

Put cellophane on top of water and see how many coins it takes to sink.

Support tubes upright in water and measure how far the water meniscus rises.

Attach small piece of blotting paper to the sensitive end of each thermometer with elastic bands, having first wetted

one of the pieces of blotting paper.

Blow on the paper and compare temperatures.

Report on how particular water property (buoyancy, surface tension, capillarity, latent heat of vapourisation) might be of use to living organisms.

Set work

Suggest improvements of the investigations that you carried out.

Notes:

Session:

1.2 Cells

Check List

- ▼ Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells.
- ▼ 'Typical' cells would be those considered to show features common to the vast majority of cells in animals or plants
- ▼ A squamous (pavement) epithelium cell from the lining of the mouth could be considered as a 'typical' animal cell.
- ▼ A mesophyll cell from a leaf could be considered as a 'typical' plant cell.
- ▼ Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body.
- ▼ In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplasts.

Materials

- ## Procedure

Make LP and HP drawings of the plant and animal cells. Using the OHTs for guidance.

Note the major differences:

- a)** in what you actually see;
- b)** what you know from your theory studies.

Explain the reasons for differences between (a) and (b)

Set Work

Write a brief justification of 'interpretation' in Biological drawing, e.g. drawing the plant cell wall as a double line (which is not actually seen down the

typical lab microscope).

Notes:

Core Principles

1.2 Cells

Cell ultrastructure

Topic Guide

Session:

Check List

- ▼ The plant cell wall is made of a fibrous cellulose framework mixed with other materials, notably pectin sticking adjacent cells together as the middle lamella.
- ▼ When stretched around an expanded (turgid) cell, the cell wall makes a considerable contribution to the support of non-woody structures, e.g. leaves. In 'woody' parts the cell wall is impregnated with lignin.
- ▼ Plasma (cell surface) membrane controls the entry and exit of all materials into and out of all cells. It is fatty in nature and contains proteins and other substances. It is partially permeable, being more permeable to water, than to dissolved solutes.
- ▼ The nucleus is surrounded by a double membrane, the nuclear envelope. Genes in the nuclear chromosomes direct all the cell's activities and determine its life span and reproductive cycle. The chromosomes are only formed just before nuclear and cell division.
- ▼ Mitochondria (singular = mitochondrion) carry out aerobic respiration, which transfers energy from energy rich substances such as glucose, into the energy rich substance ATP. High energy consuming tissues like those in the muscles or brain may contain up to a thousand mitochondria in a single cell.
- ▼ Chloroplasts are the centres of photosynthesis. They contain the green pigment chlorophyll which absorbs light for photosynthesis in which light energy is used in the synthesis of organic compounds (e.g. glucose) from carbon dioxide and water.
- ▼ Rough endoplasmic reticulum is a maze of membrane bound tubules and layers of flattened sacs enclosing a fluid filled interior, which acts as transport and storage system within the cell. It has ribosomes associated with it which are the sites of protein synthesis.
- ▼ The smooth ER tends to be more tubular, and appears to have a complex set of functions, including fat metabolism.
- ▼ Golgi body is more clearly seen in animal cells and collects, processes and redirects proteins and fats made in the rough ER. Materials are transported between the ER, the Golgi apparatus, and the plasma membrane, sealed in membrane sacs called vesicles.

Student Activity

Materials

- Electronmicrographs of animal and plant cells
- OHT Animal and plant cells as seen under the EM

Procedure

With the aid of □ OHTs **Animal** and **plant** cells as seen under the EM, interpret actual electronmicrographs.

Measure organelles on the electronmicrographs and calculate the actual size by dividing by the magnification.

Set Work

List the cell organelles in order of decreasing size.

Notes:

Session:

Prokaryotic and Eukaryotic cells

- ▼ Ribosomes present in both prokaryotes and eukaryotes.
- ▼ Prokaryotic cells may form colonies but never multicellular organisms/tissues
- ▼ Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously.
- ▼ Eukaryotic cells typically form tissues in

- Slides of bacteria, and of squamous epithelium
- OHT Prokaryotic cell

Observe the two slides under the microscope.

Make a drawing of the squamous epithelium cells and then draw the bacteria as seen down the light microscope ON the drawing of the squamous epithelium cells

Comment on their relative sizes and relate this to the fact that many bacteria are disease causing (pathogenic).

Compare what you have seen down the microscope with the $\square$ OHT Prokaryotic cell, and comment on the relative resolution and magnification of the electron microscope when compared to your light microscope.

Much of the original work on the function of DNA was done on prokaryotic cells, write a paragraph suggesting difficulties in extrapolating the findings of this work to eukaryotic cells.

Notes:

Topic Guide

Session:

Core Principles

1.2 Cells

Electron microscopy and differential centrifugation

Check List

- ▼ Electron microscopy reveals much greater detail of cell structure than the light microscope.
- ▼ Resolution is the ability to see detail. The higher the resolution the more of the detail present can be seen.
- ▼ Magnification is enlarging in size. Increasing magnification only reveals extra detail if the detail can be resolved.
- ▼ Resolving power of light microscope limited by the wavelength of light. Put simply some detail is so small that it cannot be 'hit' by light.
- ▼ Endless magnification of the image produced by a light microscope yields no extra information.
- ▼ Wavelengths of electron beams much shorter than that of light and give the electron microscope much greater resolving power than the light microscope.
- ▼ Therefore extra magnification up to $\times 250\,000$ yields an equivalent amount of detail.
- ▼ The risk of distortion and the production of artefacts is greater with the EM as a result of the total dehydration, very thin sectioning, staining, and high temperatures generated.
- ▼ It has been argued for example that the rough or granular endoplasmic reticulum is an artefact caused by the cracking and splitting of the cytoplasm pushing the ribosomes to the edge.
- ▼ Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section
- ▼ The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells.
- ▼ The transmission electron microscope beams electrons through very thin specimens
- ▼ Differential centrifugation allows the isolation of the different organelles. Mechanical disruption of the cells produce subcellular fragments. The fragments are separated by a series of spins in a high speed centrifuge (ultracentrifuge) in a process called differential centrifugation (or 'cell fractionation').
- ▼ The heaviest components separate at the lowest spinning speeds, but centrifuged 'organelle bands' not completely 'pure'.
- ▼ Lysosomes poorly identifiable in electron micrographs, identification almost entirely on functional criteria.
- ▼ Cells specialised for particular functions show organelles developed in similar way, e.g. active voluntary muscle, and secretory cells, have increased number of larger mitochondria.
- ▼ Smooth endoplasmic reticulum associated with a wide range of metabolic functions suggesting that its identification as a specific organelle is unlikely.
- ▼ Function of chloroplasts easily established with the use of the light microscope, ie recognising cell fraction that carries out photosynthesis.
- ▼ There are still major problems in correlating specific functions with clearly identifiable organelles.

Student Activity

Materials

- Newspaper photographs and Microscopes
- Laboratory centrifuge and a mixed suspension of particles with different mass (Chemistry Dept.)

Procedure

Observe newspaper photographs under the light microscope using reflected light, with increasing magnifications.

Spin down the suspension and try to sample each layer and observe under the microscope, in an attempt to simulate differential centrifugation.

Set work

By reference to your observations of the newspaper photographs under the microscope, explain the difference between resolution and magnification.

Core Principles

1.2 Cells

Cell differentiation

Topic Guide

Session:

Check List

- ▼ The cells of multicellular organisms may differentiate and become adapted for specific functions.
- ▼ In multicellular organisms cells are organised into tissues. A tissue being an association of similar cells specialised and coordinated to a particular function. Some functions being more specialised than others.
- ▼ Cells in the same tissue develop from the same original cell type, even though the fully differentiated cells may appear very different.
- ▼ Examples of tissues in mammals include epithelium covering surfaces e.g. columnar epithelial cells lining the small intestine.
- ▼ An example of a tissue in flowering plants is the palisade mesophyll found under the upper surface of the leaf
- ▼ The leaf palisade mesophyll cell is specialised for the purpose of photosynthesis. It contains numerous chloroplasts which, if living cells are observed, can be seen moving within the cytoplasm to positions favouring optimum light absorption.
- ▼ Columnar epithelial cells are specialised for the purpose of transport and the membrane across which absorption occurs is adapted to provide a huge surface area by the possession of extensions called microvilli.
- ▼ Different tissues become 'organised' into organs specialised to particular functions, e.g. a lung is an organ and consists of squamous epithelium, ciliated epithelium, connective tissue, blood vessels etc.

Student Activity

Materials

- Microscope
- Slides of T.S. small intestine and T.S. Leaf
- ☐ OHT small intestine
- ☐ OHT leaf

Procedure

Examine the slides of the animal and plant tissues, and make annotated drawings relating structure to function.

Set Work

List as many tissues and organs in the human body as you can

Notes:

Core Principles

1.3 Cell transport

Plasma membranes

Topic Guide

Session:

Check List

- ▼ All cell membranes are thought to share the same structure (except the inner membrane of mitochondria).
- ▼ This is based on a lipid bilayer formed from phospholipids aligning themselves with their hydrophobic poles next to each other within the membrane, and hydrophilic poles facing outwards.
- ▼ This fluid arrangement is stabilised by the presence of another lipid - cholesterol.
- ▼ Various proteins are embedded within this lipid bilayer.
- ▼ Extrinsic proteins are found on the surface of the membrane.
- ▼ Intrinsic proteins have one end embedded in the lipid bilayer and the other protruding out of the external surface of the membrane.
- ▼ Transmembrane proteins span the lipid bilayer and create aqueous channels of pores through which water soluble substances can pass by diffusion.
- ▼ Short polysaccharide chains attached to proteins (glycoproteins) and to fats (glycolipids) protrude from the external surface and act as cell recognition sites (receptors or antigens).
- ▼ The patchwork arrangement of all these types of molecules in the lipid bilayer is the origin of the description 'mosaic'.
- ▼ The cell surface membrane defines the limits of the cell.
- ▼ It receives 'messages' by providing receptor sites for hormones, cell recognition of 'self' and 'non-self', and act as antigens with which antibodies can bind.
- ▼ It acts as a partially permeable membrane regulating the transport of substances across the membrane.
- ▼ Internal membrane systems provide a surface for attachment of organelles and enzymes, and compartmentalise specialised functions within the cell.

Student Activity

Materials

- ☐ OHT Phospholipids and the bilayer
- ☐ OHT The fluid mosaic model

Procedure

☐ OHT Phospholipids and the bilayer explains the structure of cell membranes, building up the fluid mosaic model from the phospholipid bilayer. Use this OHT as a back transparency for drawing in first cholesterol (slows down lateral movement of phospholipids and also makes the membrane less permeable), then the three types of protein, extrinsic, intrinsic, and transmembrane. Finally draw in glycoproteins and glycolipids.

- ☐ OHT summarises the final structure.

Place some well washed identical slices of beetroot (washed until no more pigment is released) in beakers of water at a range of temperatures from 0°C to 100°C; and in a range of dilutions of detergent, and observe the release of pigment from the slices. Use a colorimeter if available.

Set work

Tabulate the results from the experiment with beetroot slices, and comment on the results.

Notes:

Core Principles

1.3 Cell Transport

Diffusion

Osmosis

Active transport

Topic Guide

Session:

Check List

- ▼ Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores.
- ▼ Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move.
- ▼ At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative).
- ▼ Facilitated diffusion occurs via specialised carriers in the membranes which reduce the resistance to the diffusion of these substances.
- ▼ Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient.
- ▼ Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.

Student Activity

Materials

- Variety of plant material
- 1M sucrose solution
- Methylene blue
- Practical Work Sheet:
The water potential of plant cells

Procedure

Investigate the water potential of a range of plant material: potato chips, beetroot (which allows for a check on any losses from damaged cells), onion rings, banana slices of different ripeness.

Investigate any alterations of density of bathing solution by colouring with methylene blue and releasing a drop of this carefully at mid-depth into a beaker of the original solution, if water has entered the cells by osmosis the bathing solution will have become more concentrated, and will sink when introduced into original solution. If the bathing solution has gained water from the cells it will be less dense than the original and rise when introduced into the original.

Osmosis and diffusion can be studied in the same investigation if solutions of urea are used. Initially plant material loses water to concentrated urea

solution and shrinks and loses mass, but after a period urea diffuses into the plant material which recovers size and mass. A puzzling and interesting phenomenon to observe.

Set Work

Write a paragraph explaining osmosis in terms of the diffusion of water.

Notes

Topic Guide

Session:

Core Principles

1.4 Exchange of materials with the environment

Surface area to volume ratio

Check List

- ▼ Surface area increases with size but surface area to volume ratio decreases.
- ▼ SA/V increased by flattened body shapes and structures.
- ▼ Where SA/V sufficiently large, simple diffusion suffices for exchanges with environment and internal transport.
- ▼ Discussions of development of transport systems also involve level of complexity of organism.
- ▼ Development of transport systems involves pumping hearts and tubes.
- ▼ Development of transport systems involves development of specialised exchange surfaces with large surface areas.
- ▼ Diffusion still important in all exchanges, but not in transport throughout volume of organism.

Student Activity

Materials

- 3 pieces of paper per student sized A4, A5 and A6
- Adhesive tape and scissors.
- OHT Cubes 2 cm and 1 cm sides

Procedure

Make three cylindrical 'animals' from the different sized pieces of paper. Roll the paper in each case taping the short ends together to make three 'animals' of similar shape and different size. Now calculate the surface area to volume ratio, making out a table like this:

Surface area (mm ²)	volume of cylinder ($\pi r^2 \times \text{length}$)	surface area /volume (mm ³)

What trend can you recognise? Untape the A4 paper and roll it up lengthwise so that it makes a longer, thinner 'animal'. Calculate the surface area/ volume ratio of this and compare it to that of the other A4 'animal'. What is the difference? Why is there a difference?

Make up two sets of jelly cubes of different size but from the same volume of jelly with a soluble dye (methylene blue). Wash well, place in water, and observe release of dye. Relate your observations to surface area/volume ratio considerations.

Now relate all this to the question of obtaining nutrients and oxygen in solution, getting rid of soluble waste etc. i.e. exchanging materials with the outside environment (assume it's an aquatic 'animal'). You should come to see that the smaller the surface area/volume ratio, the greater the need for a transport system.

What factors other than size affect the need for a transport system? Consider the importance of shape (see results of the A4 cylinders) and the level of activity, i.e. the rate of exchange needed.

Set work

Write 10 lines on 'Specialised exchange surfaces in the human body'.

Notes:

Session:

1.4 Exchange of materials with the environment

Ventilation

- ▼ Ventilation mechanisms move the external medium, e.g. air or water over the internal gas exchange surfaces, by altering the internal pressure.
- ▼ In fish and mammals the ventilation mechanisms involve muscular contractions which serve to maintain a constant flow of water or air over the gas exchange surfaces.
- ▼ The process of air or water intake is called inspiration and the expulsion of air or water is called expiration.
- ▼ Bony fish have a double pump system as a result of the actions of the floor of the pharynx and the opercula flaps.
- ▼ Water enters the mouth when the internal pressure is reduced below the external water pressure, and pumped out when it is greater.
- ▼ These pressure changes are achieved by alternating pumping movements of the floor of the buccal cavity and the opercula. These pumps alternate to produce a continuous one way flow of water over the gills,
- ▼ With no danger of dehydration by evaporation over the thin delicate gas exchange surfaces of the gills, they can be fully exposed to the external medium.

- ▼ With the lungs of terrestrial animals there is a need to maintain a humid atmosphere over the thin delicate gas exchange surfaces so they are enclosed interiorly.
- ▼ Ventilation produces tidal flow in and out of a single aperture. This is less efficient than the one way flow in fish gills, as the air is not completely renewed in every breathing cycle.
- ▼ Inspiration (breathing in) - the external intercostal muscles contract, pulling the ribs upwards and outwards, the diaphragm contracts, moving downwards, the volume of the thorax increases, the pressure in the thorax decreases below atmospheric pressure causing air to enter, inflating the lungs.
- ▼ Expiration (breathing out) depends upon the elasticity of the tissues of the lungs and thorax. The respiratory muscles relax, the thorax and lungs recoil as a result of their elasticity and the events of inspiration are reversed.
- ▼ The elasticity of the the tissues of the lungs and thorax leads to expiration as they recoil.

Notes:

- Bony fish
- Model of mammalian thorax and contents
- Bell jar model of diaphragm movements

The two systems can be directly compared (with models or dissections, bony fish gill dissection best carried out under water)

Describe the ways in which fish gills can be considered as more efficient than mammalian lungs.

Core Principles

1.5 Enzymes

Action of enzymes

Topic Guide

Session:

Check List

- ▼ All enzymes are protein in nature, and fall into the group of 'globular' proteins with a complex 3D structure.
- ▼ Enzymes are catalysts which speed up the rate of reaction of much larger amounts of reactants without themselves being used up in the process.
- ▼ Unique amongst catalysts they are denatured by extreme conditions of pH and temperature etc.
- ▼ Their activity depends upon their complex 3D shape which creates an active site which combines with the substrate to form an enzyme/substrate complex which lowers the activation energy of reactants.
- ▼ Their complex 3D shape also accounts for enzyme specificity as the active site has a shape complementary to the shape of the substrate.
- ▼ Disruption of the 3D shape accounts for denaturation.
- ▼ pH has an effect on the 3D shape of enzymes, especially the active site which has an electric charge.
- ▼ Any effect on the active site alters the reactivity of the enzyme. There is an optimum pH, either side of which reactivity drops.
- ▼ Extremes of pH permanently disrupt the active site and thus denature the enzyme.
- ▼ Similar effects are seen with temperature, except low temperatures do not denature enzymes. The rate of enzyme catalysed reactions, like all chemical reactions, are reduced with low temperatures.
- ▼ Optimum conditions of pH and temperature vary with the particular enzyme, which reflects the conditions in which the cell exists.
- ▼ The relative amounts of enzyme and substrate affect the rate of reaction as it affects the formation of the enzyme substrate complex. Only one can be rate regulating or limiting at any one time.

Student Activity

Materials

- Practical Work Sheet:
The effect of temperature on the activity of Catalase
- ☐ OHT Temperature, pH, enzyme and substrate concentration
- ☐ OHT Activation energy
- ☐ OHT Enzyme-substrate complex

Procedure

- ☐ OHT Activation energy discussion of activation energy and catalysts. Define 'enzyme'. Explain that enzymes are proteins. Use the opportunity to revise tertiary structure, stress the importance of shape.
- ☐ OHT Enzyme-substrate complex to illustrate active site, enzyme-substrate complex, specificity. Define these terms and make notes.

Investigate the effect of temperature on the activity of catalase.

Set work

Plot the curve for the rate of a typical chemical reaction against temperature from 0 - 100°C

On the same axes plot the rate of an enzyme catalysed reaction with an optimum temperature of 40°C.

Explain the relationship shown by the two curves.

Notes:

Core Principles

1.6 Digestion

Extracellular digestion

Topic Guide

Session:

Check List

- ▼ Digestion is the breaking down of larger food molecules into smaller absorbable molecules by the action of enzymes.
- ▼ Digestion may occur outside (extracellular digestion) or inside (intracellular) the body of living organisms.
- ▼ Fungi secrete digestive enzymes onto a food substrate and the products of digestion are absorbed by diffusion.
- ▼ It should be noted that term extracellular digestion simply means 'digestion outside the cell' so the action of many enzymes in the mammalian gut may also be described as extracellular. In the context of fungi it is often taken to mean 'outside the body' rather than just the cells.
- ▼ Starch agar plates can be used to assess the activity of (assay) carbohydrase enzymes.
- ▼ Carbohydrase enzymes (e.g. amylase) or substances containing carbohydrase enzymes such as germinating seeds are placed in each well.
- ▼ Carbohydrase enzymes diffuse from the wells into the surrounding starch-agar mixture and begin to digest the starch.
- ▼ The plate is then flooded with iodine in KI solution and the size of clear zones after a given time period can be used as a measure of the rate of enzyme catalysed reaction.

Student Activity

Materials

■ Practical Work Sheet:

The action of amylase on starch agar plates

□ Starch agar plates

Procedure

Carry out the starch agar / amylase experiment 'assaying' the activity of different concentrations of amylase.

Set Work

Record the results of the experiment in a table and plot a suitable graph from which solutions of unknown concentration can be assayed.

Notes:

Core Principles

1.6 Digestion

Digestion in humans

Topic Guide

Session:

Check List

- ▼ Enzymes are produced in four main locations within the intestine:
 - From glands within the mucosa and submucosa
 - From large glands outside the gut wall
 - On the surface of epithelial cells of the small intestine mucosa
 - Inside epithelial cells of the small intestine mucosa
- ▼ The sites of production and action of amylases, endopeptidases, exopeptidases, lipase, maltase, and bile ensure digestion before the end of the small intestine.
- ▼ The action of salivary amylase continues for a short time in the stomach but is denatured by the stomach acid.
- ▼ Endopeptidases hydrolyse peptide bonds within proteins producing smaller polypeptides, and are secreted by the stomach and pancreas.
- ▼ Exopeptidases hydrolyse terminal peptide bonds at the ends of proteins releasing amino acids, and are secreted by the cells of the ileum and pancreas.
- ▼ Lipase digests lipids and is secreted by the pancreas.
- ▼ Bile contains no enzymes but provides alkaline conditions and salts that emulsify fat, and is indispensable to the digestive process.
- ▼ Maltase digests maltose to glucose, and is located in the cell membrane of the cells of the ileum.
- ▼ The end products of digestion enter the epithelial cells of the ileum and by simple diffusion, facilitated diffusion, active transport and exocytosis.
- ▼ Diffusion is the movement of substances from a high concentration to a lower concentration. No energy and no carrier molecules are required.
- ▼ Facilitated diffusion is the movement of substances from a high concentration to a lower concentration using specific protein carrier molecules or protein channels, no energy is required.
- ▼ Active transport is the movement of substances against their concentration gradient (from low to high) via protein carrier molecules. Energy in the form of ATP from respiration is required.
- ▼ Exocytosis occurs when substances inside vesicles are removed from a cell when the membrane of the vesicle fuses with the cell surface membrane.

Student Activity

Materials

- Microscopes and slides of sections of the mammalian gut
- OHT Human gut

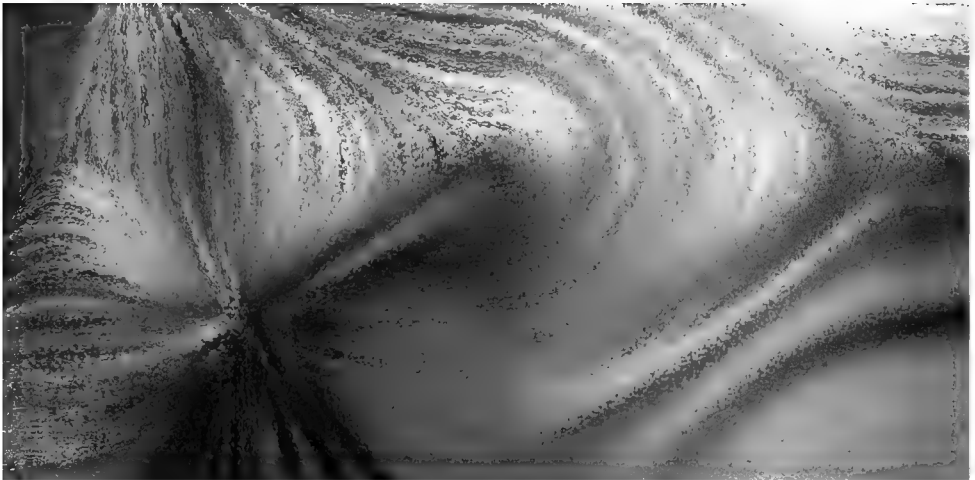
Procedure

Relate the sections seen under the microscope to the regions of the whole gut.

Set Work

Write a paragraph explaining the problems involved in the gut secreting protein digesting enzymes.

Notes:



Topic Guide

Session:

Genes and Genetic Engineering

2.1 The genetic code

The gene Structure of DNA Replication of DNA

Check List

- ▼ A gene is a length of DNA which carries the genetic information for the synthesis of a single polypeptide chain. The gene determines the primary structure of the polypeptide, that is the sequence of amino acids.
- ▼ Polypeptide chains form proteins, all enzymes are proteins, and enzymes determine the nature and development of organisms.
- ▼ A change (mutation) may occur in the genetic code in the gene, and this changed form of the gene is known as an allele of that gene.
- ▼ Each gene occurs in one particular position on a specific chromosome known as its locus. Therefore where there are two sets of chromosomes (diploid) in a nucleus, the chromosomes occur in pairs each one of which carries the same genes at the same loci. The chromosomes are described as homologous.
- ▼ DNA and RNA are long chain polynucleotide molecules with repeating sub-units of nucleotides.
- ▼ Nucleotides consist of a 5 carbon sugar, a phosphate and an organic base.
- ▼ In DNA the sugar is deoxyribose and the organic base can be one of adenine, thymine, cytosine and guanine.
- ▼ In RNA the sugar is ribose and the organic base can be one of adenine, uracil, cytosine and guanine.
- ▼ The DNA molecule is a double stranded helix with the two strands held together by hydrogen bonds between complementary base pairs adenine-thymine and cytosine-guanine.
- ▼ RNA is a single stranded helix with base pairs of adenine-uracil and cytosine-guanine.
- ▼ There are three types of RNA - messenger, transfer and ribosomal.
- ▼ DNA carries out semi-conservative replication in which each strand of the original molecule acts as a template for the construction of a new one, so that each new double stranded molecule of DNA consists of one original strand and a new complementary strand.
- ▼ The synthesis of new strands of DNA is catalysed by enzymes, including DNA polymerase.

Student Activity

Materials

- DNA molecular model
- OHTs showing structure of DNA, RNA and semi-conservative replication (with DNA polymerase)

Procedure

Use the 3D model of DNA structure to clarify any problems with envisaging the 3D structure from 2D diagrams.

Set work

What does 'semi-conservative replication' mean? Explain a piece of experimental evidence for semi-conservative replication.

Notes

Genes and Genetic Engineering

2.1 The genetic code

The genetic code

Topic Guide

Session:

Check List

- ▼ The sequence of nucleotide bases on one strand of the DNA molecule carries the genetic code for the synthesis of proteins and hence ultimately living organisms.
- ▼ Three bases (a triplet codon) code for one amino acid.
- ▼ There are 64 (4^3) possible combinations of any three from four bases, but only 20 amino acids to be coded for.
- ▼ Amino acids are coded for by more than one triplet codon and the code is said to be 'redundant' or 'degenerate'.
- ▼ A gene is a sequence of nucleotide bases of a DNA molecule which codes for a polypeptide.
- ▼ A gene for a polypeptide has a specific 'start' codon which thus sets the order of all the subsequent triplet codons
- ▼ Thus the code is non-overlapping i.e. a base can only be 'used' in one triplet codon.
- ▼ Only a fraction of the nuclear DNA contains genes coding for proteins, the rest consists of so-called 'junk' DNA the function of which is not understood.

Student Activity

Materials

- ☐ OHT showing base sequence / gene code

Procedure

Use OHTs to revise and discuss the genetic code

Set work

Draw out a random sequence of bases of a strand of DNA and annotate it to demonstrate the non-overlapping nature of the genetic code.

Draw out the complementary strand of DNA to the one that you have drawn and annotate to illustrate how only one strand can carry the genetic information.

Notes:

Session:

Role of nucleic acids in protein and enzyme synthesis

- ▼ Nuclear DNA with the genetic code never leaves the nucleus in eukaryotic cells.
- ▼ The genetic code (a gene) for a polypeptide is copied (transcribed) into mRNA which leaves the nucleus and associates with the ribosomes.
- ▼ The ribosomes 'read' the genetic message on the mRNA, and tRNA delivers amino acids in the sequence determined by the code.
- ▼ There are 20 different types of tRNA, each specific to a particular amino acid.
- ▼ Each tRNA has a specific binding site at which the specific amino acid combines and an anti-codon which matches the specific codon for that particular amino acid.
- ▼ The amino acids combine by means of peptide bonds to give a polypeptide chain.
- ▼ The polypeptides subsequently form proteins.
- ▼ As enzymes are proteins their synthesis is controlled by DNA.

Notes:

- ❑ OHTs Transcription and translation
- ❑ OHT Table of amino acid codons

- ❑ OHT Transcription and translation
- ❑ OHT Table of amino acid codons

Can be used to revise transcription and translation.

DNA replication, transcription, and RNA translation all require enzymes to catalyse their reactions. Explain the particular problem that this represents in terms of protein synthesis.

Genes and Genetic Engineering

2.1 The genetic code

Mutation

Topic Guide

Session:

Check List

- ▼ A change (mutation) may occur in the sequence of bases in a gene, and this changed form of a gene is known as an allele of that gene.
- ▼ Mutations of a gene may occur many times in members of a species so there may be many alleles of a gene (multiple alleles) in a population, but each diploid organism can only ever have two alleles of a gene, at one particular locus on a pair of homologous chromosomes.
- ▼ The type of mutations that occur can include additions, deletions, and substitutions of bases, all of which alter the sequence of bases, and consequently the sequence of amino acids in the polypeptide chain for which the gene codes.
- ▼ Additions involve the inclusion of an additional base in the DNA sequence, which has a 'knock on' effect on all subsequent triplet codons.
- ▼ Deletions involve the loss of a base in the DNA sequence, which also has a 'knock on' effect on all subsequent triplet codons.
- ▼ Substitutions involve the replacement of a base by another, this type of mutation only affects one triplet codon, and consequently only one amino acid in the polypeptide chain (the fact that an amino acid is coded for by more than one triplet codon means that a substitution may not alter the amino acid sequence).
- ▼ Mutations occur naturally at random, but many factors (mutagenic agents) increase the rate of mutation.
- ▼ High energy radiation such as X rays, gamma rays, and ultra violet light cause ionisations in DNA molecules. The changed electrical charges alter the shape and therefore the function of DNA.
- ▼ High energy particles such as alpha particles, beta particles (electrons) and neutrons all damage DNA as they pass through the nucleus.
- ▼ Some chemicals such as benzene, xylene, nitrosamines, mustard gas, tar in tobacco smoke etc cause mutations.
- ▼ Many mutations lead to cancer, and agents that cause cancer are known as carcinogenic.
- ▼ Alterations in the polypeptide chain affect the secondary and tertiary structures of the final protein upon which its activity depends.
- ▼ A non-functioning enzyme can cause a metabolic block to occur in a metabolic pathway.

Student Activity

Materials

- ☐ OHT Gene mutations

Procedure

- ☐ OHT Gene mutations can be used to illustrate the three types of mutation mentioned above.

Set Work

Write a paragraph differentiating between the terms gene and allele.

Notes:

Genes and Genetic Engineering

2.2 The cell cycle

Mitosis

Topic Guide

Session:

Check List

- ▼ Mitosis is nuclear division in which the daughter nuclei have the same chromosome number as the parent nucleus, typically diploid nuclei producing diploid daughter nuclei.
- ▼ The daughter nuclei are therefore genetically identical to the nucleus of the cell that produced them (except for any mutations that may occur).
- ▼ Mitotic cell division is seen in growth, repair and asexual reproduction.
- ▼ As a result of mitosis in the growth of an organism from the fertilised egg all cells therefore are genetically identical.
- ▼ Similarly, offspring of asexual reproduction lack genetic variation.
- ▼ The production of genetically identical cells with the full set of genetic information allows the replacement of any cells in repair processes. In the asexual reproduction of organisms it allows for the rapid exploitation of optimum conditions where any variation would be disadvantageous.
- ▼ The DNA replicates in interphase so that in the first stage of mitosis (prophase) the chromosomes appear as double structures of two chromatids (daughter chromosomes) joined at the centromere.
- ▼ The nuclear spindle apparatus(NSA) forms from the dissolution of the nuclear membrane and the centrioles replicate and migrate to either pole of the NSA.
- ▼ The centromeres align on the equator of the NSA (metaphase).
- ▼ The centromeres divide and the chromatids are moved to the opposite poles (anaphase).
- ▼ Two new daughter nuclei are formed (telophase).

Student Activity

Materials

- Microscopes
- Slides of root tip squashes
- Supplementary Practical Sheet:
Preparation of root tip squash to show stages of mitosis
- OHT Root tip squash
- OHTs Normal Human Karyotype OHTs-35-36-37-38

Procedure

Observe slides of root tip squashes.

Make drawings of prophase, metaphase, anaphase and telophase nuclei.

Look for similarities between the root tip cells and the human nucleus.

Set work

Determine at what stage in mitosis the photograph of the human nucleus and chromosomes was taken,

and from what type of cell. Explain your reasons.

Notes:

Genes and Genetic Engineering

2.2 The Cell Cycle

Applications of cloning

Topic Guide

Session: _____

Check List

- ▼ Clones are genetically identical organisms.
- ▼ Cloning may occur naturally, for example, identical twins in humans, binary fission in microorganisms and vegetative reproduction in plants, but it can also be achieved artificially.
- ▼ The production of crops by vegetative propagation e.g. in which cuttings are taken from a desirable original is a form of cloning, some common crop plants, for example the banana, can only be grown by cloning.
- ▼ Since members of a clone are genetically uniform under optimum conditions, they grow to the same height, flower at the same time, and produce fruit of a similar size, features which make them highly suitable for mechanised farming.
- ▼ The uniformity which makes them so attractive to farmers applies also to their susceptibility to pests, diseases and other environmental hazards. An entire crop may be wiped out by a single type of pathogen.
- ▼ It is now possible to clone plants from cell cultures through the techniques of micropropagation. In some cases, whole new plants can be propagated from single cells.
- ▼ This is useful in producing virus free stock from apical meristem cells which viruses do not penetrate.
- ▼ The cloning of animals is possible by new techniques for manipulating embryonic cells, e.g. by splitting apart the cells of developing embryos at a very early stage after fertilisation.

Student Activity

Notes: _____

Materials

- Samples of material illustrating vegetative reproduction by plants.

Procedure

Examine the plant material to illustrate vegetative reproduction and make annotated sketches.

Set Work

Write a paragraph explaining the role of mitosis in cloning.

Genes and Genetic Engineering

2.3 Sexual Reproduction Gametes and fertilisation Meiosis Importance of meiosis

Topic Guide

Session:

Check List

- ▼ In sexual reproduction DNA from one generation is passed from one generation to the next by sex cells (gametes) which fuse their nuclei in fertilisation.
- ▼ Male gametes are smaller, much more numerous, and motile, finding their way to the fewer, larger, immobile female gamete(s) in a variety of ways.
- ▼ The male gamete contributes only its nucleus in the process of fertilisation, the cytoplasm of the female gamete is vital in the development of the fertilised egg into a multicellular organism.
- ▼ Each gamete of a sexually reproducing organism contains one set of chromosomes (haploid).
- ▼ When two such gametes fuse in fertilisation they produce a zygote with two sets of chromosomes (diploid).
- ▼ Chromosomes in a diploid nucleus thus occur in pairs, each member of a pair carrying the same genes in the same relative positions (homologous chromosomes).
- ▼ The production of haploid gametes from a diploid cell requires a reduction division (meiosis) in which the chromosome number is halved from diploid to haploid.
- ▼ The production of haploid gametes ensures the maintenance of the diploid chromosome number in subsequent generations.
- ▼ Life cycles of sexually reproducing organisms therefore involve mitosis, meiosis, fertilisation and changes in chromosome number.
- ▼ Animals and flowering plants are diploid with meiosis forming haploid gametes, but mosses and their relatives are haploid, gametes are produced without meiosis, and meiosis precedes spore formation. The haploid spores form new haploid moss plants.

Student Activity

Notes:

Materials

- Microscope and slides of meiosis in plant and animal tissue
- OHT Meiosis

Procedure

Make annotated sketches of the stages of meiosis

Set Work

Make a table of the similarities and differences between mitosis and meiosis.

Genes and Genetic Engineering

24 Applications of gene technology Principles of genetic engineering The polymerase chain reaction Genetically engineered microorganisms Genetic markers Large scale culturing

Topic Guide

Session:

Check List

- ▼ Genetic engineering is the manipulation of the DNA of an organism. It primarily involves removing genes from one organism (the donor) and inserting them into another organism (the recipient or host). The recipient organism is known as a transgenic organism.
- ▼ There are three basic stages:
- ▼ The desired gene must be located in the DNA of the donor organism or created from the mRNA of a known protein.
- ▼ The desired gene must be removed from the DNA of the donor using restriction enzymes which cut DNA at specific points and allow removal of a small section.
- ▼ The desired gene must be inserted into the DNA of the recipient organism. Having cut the DNA of the recipient organism using restriction enzymes, ligase enzymes are used to join the new gene permanently into its own DNA.
- ▼ The Polymerase Chain Reaction (PCR) is a technique used to amplify (make many copies of) a DNA molecule or part of a DNA molecule.
- ▼ PCR has revolutionised genetic technology. It theoretically allows any number of copies of DNA to be produced from a single DNA molecule (the template).
- ▼ PCR, radioactive labelling and electrophoresis in which fragments of different size are separated and identified allows the determination of the sequence of nucleotides in DNA.
- ▼ Genetically engineered microorganisms include many different types of bacteria and more recently some types of yeast
- ▼ Plasmids are often used as vectors to incorporate selected genes into bacterial cells.
- ▼ To check which bacteria from the clones have been transformed (and are hence useful for producing a product) a special technique involving marker genes for antibiotic resistance is often used.
- ▼ Bacteria transformed by recombinant plasmids being resistant to ampicillin but susceptible to tetracycline.
- ▼ Large scale culturing of genetically modified microorganisms involves techniques to maximise bacterial growth and hence production
- ▼ Once established vessels up to 200 000 litres may be used. With fermenters of this size, several hundred tonnes of product can be generated per day.
- ▼ There are two main types of fermentation: batch fermentation and continuous culture. Batch fermentation is where a single culture of microorganisms and a single batch of nutrient medium is used to produce one batch of product.

Student Activity

Materials

□ OHTs Applications of gene technology

Procedure

Concentrate on the specification headings as set out above to navigate through this difficult topic. A thorough knowledge of DNA transcription, and mRNA translation will be essential.

Set work

Prepare your ideas on the ethical, social and economic issues involved in the use of genetic engineering in medicine and food production.

Notes:

Topic Guide



3.1 Transport Systems

3.2 The Control of Breathing and Heartbeat

3.3 Energy and Exercise

3.4 The Transport of Substances in Plants

Physiology and Transport

3.1 Transport systems

Mass transport

Check Lis

- ▼ Surface area increases with size but surface area to volume ratio decreases.
- ▼ SA/V increased by flattened body shapes and structures.
- ▼ Where SA/V sufficiently large, simple diffusion suffices for exchanges with environment and internal transport.
- ▼ Discussions of development of transport systems also involve level of complexity of organism.
- ▼ Over large distances in organisms, efficient supply of materials is provided by mass transport (the bulk movement of substances through transport systems).
- ▼ Development of transport systems in animals involves pumping hearts and tubes.
- ▼ The transport systems of larger organisms involves development of specialised exchange surfaces with large surface areas.
- ▼ Specialised exchange surfaces in flowering plants are the leaves and roots, and in the mammal the lungs, gut, and kidneys.
- ▼ The main function of specialised exchange surfaces is to maintain concentration gradients so that diffusion is maintained in the required direction.
- ▼ Diffusion still important in all exchanges, but not in transport throughout organism.

Student Activity

Materials

- 3 pieces of paper per student sized A4, A5 and A6
- Adhesive tape and scissors.
- OHT Cubes 2 cm and 1 cm sides

Procedure

Make three cylindrical 'animals' from the different sized pieces of paper. Roll the paper in each case taping the short ends together to make three 'animals' of similar shape and different size. Now calculate the surface area to volume ratio and tabulate the results

What trend can you recognise? Untape the A4 paper and roll it up lengthwise so that it makes a longer, thinner 'animal'. Calculate the surface area/volume ratio of this and compare it to that of the other A4 'animal'. What is the difference? Why is there a difference?

Make up two sets of jelly cubes of different size but from the same volume of jelly with a soluble dye (methylene blue). Wash well, place in water, and observe release of dye. Relate your observations to surface area/volume ratio considerations.

Now relate all this to the question of obtaining

nutrients and oxygen in solution, getting rid of soluble waste etc. i.e. exchanging materials with the outside environment (assume it's an aquatic 'animal'). You should come to see that the smaller the surface area/volume ratio, the greater the need for a transport system.

Set Work

What factors other than size affect the need for a transport system? Consider the importance of shape (see results of the A4 cylinders) and the level of activity, i.e. the rate of exchange needed.

Notes:

Sessions:

Mammalian heart

- ▼ External appearance of heart includes small 'flip-like' atria, coronary blood vessels, fat deposits, and major vessels entering and leaving.
- ▼ Internal structure of two atria and two ventricles, with atria and ventricles separated by bicuspid and tricuspid valves, and the left and right sides separated by the septum.
- ▼ Chordae tendinae and muscles prevent back-opening of bicuspid and tricuspid valves (atrioventricular valves) when ventricles contract (ventricular systole).
- ▼ Pocket (semilunar) valves at base of pulmonary artery and main aorta prevent backflow of blood into

- ▼ Atria walls thinner than ventricle walls.
- ▼ Left ventricle wall thicker than right ventricle wall to pump blood around body (systemic circulation).
- ▼ Right ventricle wall thinner as only has to pump blood via pulmonary circulation around lungs, where reduced pressure prevents formation of tissue fluid in alveoli.
- ▼ Volumes of both left and right ventricles the same, as right ventricle supplies the left ventricle with blood via the lungs and left atrium.

Notes:

Physiology and Transport

3.1 Transport systems

Mammalian heart

Topic Guide

Session:

Check List

- ▼ The events of a complete filling and emptying of the heart is known as the cardiac cycle.
- ▼ It is a continuous cycle, start point of which can be taken when heart is empty and all valves shut.
- ▼ Atria fill and internal pressure rises forcing bicuspid and tricuspid valves open.
- ▼ Ventricles fill so that whole heart full.
- ▼ Atria contract (atrial systole) so that ventricles overfilled and stretched.
- ▼ Ventricles contract with a force proportional to their degree of stretch (ventricular systole).
- ▼ Blood forced up shutting bicuspid and tricuspid valves (first heart sound) and opening pocket valves as blood forced up pulmonary artery and aorta.
- ▼ Ventricles relax (ventricular diastole) blood falls back in pulmonary artery and aorta shutting pocket valves (second heart sound).
- ▼ During systole the pressure inside the heart and the arteries rises (systolic blood pressure).
- ▼ During diastole the pressure inside the heart and the arteries falls (diastolic blood pressure).
- ▼ These waves of pressure are felt as the pulse in the arteries.

Student Activity

Materials

- Blood pressure measuring device
- Stethoscope
- Practical Work Sheet:
Measuring the resting pulse rate
- ☐ OHT Graph/chart of pressure changes during the cardiac cycle

Procedure

Listen to heart sounds using stethoscope. Relate sounds to valve closures and stages of cardiac cycle.

Demonstrate the measurement of systolic and diastolic blood pressure. Demonstrate venous blood pressure

Stand back to a wall and raise arm to level with heart. At this point veins in hand and arm should still be visible (if visible in the first place). Mark this position. Get an assistant to raise the arm until veins 'collapse' mark this new position. Note the distance (d) between the two marks in cms. Calculate the venous blood pressure from:

$$(d-10) \times 1.055/13.55 \times 10 = \text{answer in mm.Hg}$$

Set work

Record your pulse rate before rising each morning and just before going to bed each evening for a week, and plot as a graph of pulse rate against time.

Notes:

Physiology and Transport

3.1 Transport systems

Blood vessels

Check List

- ▼ Arteries thick elastic walls with smooth muscle.
- ▼ Elasticity of arteries important in smoothing flow of cardiac output - the pulse
- ▼ Lined with smooth endothelium common to all blood vessels.
- ▼ Dividing into smaller branches leading into arterioles
- ▼ Capillary beds supply all tissues providing large surface areas for exchanges by diffusion and short diffusion distances.
- ▼ Capillaries have large total cross sectional surface areas. Therefore resistance to flow, blood pressure and rate of flow all drop in capillaries.
- ▼ Diameter of capillary same as red blood corpuscles thus forcing them to pass through capillaries in single file, bringing all close to tissues.
- ▼ Plasma minus proteins exuded through thin walls to bathe tissues directly.
- ▼ Thin walls and large lumen of veins offer little resistance to flow of blood back to heart, and allow ease of massage by surrounding muscles.
- ▼ Pocket valves ensure one way flow back to heart.
- ▼ Venous blood under low pressure but flow fast enough to ensure return of blood to heart at same rate as blood flow in the arteries.

Student Activity

Materials

- Microscope slides of TS artery and vein
- Microscope slides of TS alveoli and TS kidney glomeruli
- Practical Work Sheet:
The observation of sections of blood vessels under the light microscope
- OHT Structure of **arteries, veins** and **capillaries**

Procedure

TS Artery and Vein are often on the same slide.

Artery recognised by wavy black lines of elastic tissue in thicker walls in typically stained slides.

Lining often slightly corrugated as a result of shrinkage of artery during slide preparation.

Veins comparatively so large and thin walled they can be overlooked as a large space in the tissue.

Pocket valves rarely seen in TS.

Capillaries only clearly seen in section of alveoli or kidney glomeruli, both regions of large exchanges of gases and fluid respectively.

Can observe functioning capillaries in tadpole tails if right time of year (March/April).

Make line drawings of each type of vessel paying careful attention to linear magnification of final drawings.

Set work

Calculate the relative diameters of each vessel in terms of the number of times greater than that of a capillary.

Notes:

Physiology and Transport

3.1 Transport systems

Exchange of materials

Topic Guide

Session:

Check List

- ▼ The main substances transported by the blood system, and the sites at which exchange occurs relate to respiration, nutrition and excretion.
- ▼ Oxygen combines reversibly with haemoglobin to form oxyhaemoglobin in which form it is transported from the alveoli to the tissues.
- ▼ Haemoglobin loads up with oxygen at the alveoli, at the tissues higher temperatures, and high levels of carbon dioxide and lactate which lower pH encourage the dissociation of oxyhaemoglobin and the release of oxygen to the tissues.
- ▼ These events are illustrated in an oxygen haemoglobin dissociation curve which is moved to the right as the affinity for oxygen decreases.
- ▼ Carbon dioxide is transported in the blood as carbaminohaemoglobin in the red blood cells and hydrogencarbonate ions in the plasma to be released into the alveoli.
- ▼ The soluble end products of digestion: glucose, fructose, amino acids, glycerol and fatty acids are absorbed from the gut and transported to the liver where exchanges occur to optimise the blood plasma composition.
- ▼ Waste products e.g. urea are collected from the liver, transported to the kidneys where osmoregulation and excretion are carried out, with urea being excreted and the blood concentration being optimised

Student Activity

Materials

- Microscopes and slides of T.S. alveoli, T.S. small intestine and T.S. kidney.
- Handout mammalian circulation
- OHT [Mammalian circulation](#)

Procedure

Annotate the handout of the basic plan of the mammalian circulation to show the main substances transported by the blood system, and the sites at which exchanges occur.

Observe the slides and make sketches of the three structures.

Set Work

Annotate your sketches with regard to the features that exchange surfaces have in common.

Notes:

Topic Guide

Session:

Physiology and Transport

3.1 Transport systems

Tissue fluid

Check List

- ▼ The hydrostatic pressure of the blood in the capillaries causes plasma minus its proteins to be forced through the thin walls of the capillaries.
- ▼ This fluid bathes all the tissues and is known as tissue fluid.
- ▼ It carries substances in solution from the blood to supply the tissues, e.g. oxygen, nutrients, hormones, antibodies etc; and drains waste products e.g. carbon dioxide away.
- ▼ Most of the tissue fluid is reabsorbed back into the capillaries as the hydrostatic pressure drops as the blood passes through the capillaries.
- ▼ Excess tissue fluid is drained away into blind ending lymphatic capillaries.
- ▼ Lymph is tissue fluid that has been drained into lymphatic capillaries, with lymphocytes added by lymph nodes which occur at intervals.
- ▼ Special lymph capillaries in the villi of the lining of the small intestine, known as lacteals, preferentially absorb and transport fats, so that lymph contains more fats than plasma.
- ▼ Eventually the lymph is returned to the general circulation in the major veins near to the heart.
- ▼ Movement of the lymph in the lymphatic system is maintained by the massaging effects of the muscles and breathing movements, and the one-way pocket valves; all features common to the veins of the circulatory system.

Student Activity

Materials

- Slide of stained blood smear.
- OHT Relationship between blood and lymph

Procedure

Differentiate between the terms blood, plasma, tissue fluid and lymph.

Consider the blood vessels and lymph vessels of a working muscle. Discuss what might happen to the following blood components which enter via the artery:

- a) a molecule of glucose
- b) a molecule of oxygen attached to haemoglobin
- c) a molecule of water from the plasma.

Set work

Explain how the lymphatic system helps to maintain a relatively constant blood volume.

Why is this important?

Notes:

Sessions:

Control of ventilation

▼ At the end of inspiration, stretch receptors in the bronchioles send impulses to the inspiratory control centre in the medulla, which is switched off, and the diaphragm and external intercostal muscles relax. The elasticity of the the tissues of the lungs and thorax leads to expiration as they recoil.

Notes:

Physiology and Transport

3.2 The control of breathing and heartbeat

Control of heartbeat

Check List

- ▼ Cardiac muscle is myogenic.
- ▼ Rate modified by sinu-atrial node (pacemaker) in wall of right atrium.
- ▼ Waves of electrical activity spread rapidly down through cardiac muscle of atria.
- ▼ Prevented from passing directly into ventricles by barrier of connective tissue, stimulus picked up by atrio-ventricular node.
- ▼ Bundle of His composed of Purkyne tissue transmits stimulus to base of ventricles from where it radiates up through the walls back to the connective tissue barrier.
- ▼ Detected by electrodes on the skin as an ECG.
- ▼ Pacemaker influenced by sympathetic (faster) and parasympathetic (slower) nervous systems, and the hormone adrenaline.
- ▼ All influences on heart rate coordinate heart rate and therefore blood supply to body (cardiac output) with demands of body.
- ▼ Pressure receptors near entries of great veins detect amount of blood returning to heart in veins, and increase the cardiac output if venous return increases
- ▼ Pressure receptors in the aortic arch and carotid sinus measure pressure of blood being pumped out by the heart, and adjust cardiac output accordingly via the centres in the medulla of the brain.

Student Activity

Materials

- ECG charts
- OHT Diagram of heart and SA node

Procedure

Observe how the nerve message is transmitted through the heart with the aid of the OHT. Annotate a copy of this OHT

Observe copies of ECG charts (in groups) and explain how they are obtained.

Relate the ECGs to the events illustrated on the OHT.

Set work

Artificial pacemakers re-establish a fixed rhythm in those suffering erratic heart beats. Explain the effect on daily activities of having an artificial pacemaker fitted.

Notes:

Topic Guide

Session:

Physiology and Transport

3.3 Energy and exercise

Energy sources

Muscle fatigue

Check List

- ▼ The energy rich materials in the diet, namely fats, carbohydrates, and proteins, are the source of all energy within the body.
- ▼ The most important direct sources of energy within the body are glucose, glycogen and triglycerides (fats).
- ▼ The end product of all the energy releasing processes of cellular respiration is the energy rich compound adenosine triphosphate or ATP. ATP is the universal energy currency of all living things. It is the form in which energy is supplied for all purposes.
- ▼ ATP contains three phosphate groups, the end one of which can be more easily displaced than the others, with energy being made available for use by the muscle fibres. In this process, adenosine diphosphate (ADP) and a free inorganic phosphate group (Pi) are formed.
- ▼ This process is reversible, and energy released from the oxidation of energy rich materials can be trapped in the ATP molecule when a phosphate group is combined with ADP to reform ATP.
- ▼ Respiratory substrates are oxidised to carbon dioxide and water, in the process known as cellular respiration. The breakdown of the different respiratory substrates involves common pathways.
- ▼ The initial stages in the breakdown of carbohydrates do not involve oxygen and they are therefore referred to as anaerobic glycolysis.
- ▼ Anaerobic glycolysis generates a net gain of two ATP molecules from each molecule of glucose, which only represents about 5% of the total ATP that can be generated from the complete breakdown of the glucose molecule.
- ▼ If sufficient oxygen is available the pyruvic acid is completely oxidised into carbon dioxide and water, and the production of many more molecules of ATP.
- ▼ These aerobic stages take place in the mitochondria.
- ▼ When the demand of the muscles for oxygen exceeds the supply, lactate begins to accumulate in the blood.
- ▼ The increased acidity (decreased pH) as a result of the production of lactate has a complex of effects, all of which contribute to fatigue. It inhibits resynthesis of creatine phosphate, and interferes with muscle contraction. It also inhibits enzymes, especially those involved in glycolysis, and decreases the amount of energy released by the hydrolysis of ATP.
- ▼ The major pathway for the removal of lactate is its aerobic oxidation to carbon dioxide and water, yielding ATP mainly in the heart, brain, liver, and kidneys.

Student Activity

Materials

□ OHT ATP

Procedure

Review anaerobic and aerobic pathways but do not get bogged down in discussions of how many molecules of ATP are generated per molecule of glucose in the different pathways. The important point is the relative amounts.

The fatiguing effects of lactate can be demonstrated by holding the non-writing arm in the air, and rapidly clenching and reclenching both fists. The raised arm has a restricted blood supply as a result of gravity, and the lowered arm has a normal supply.

The raised arm soon seizes up with lactate accumulation and fatigue, whilst the lowered arm can continue indefinitely.

Set Work

Make a sketch of a mitochondrion and add labelled arrows showing the movement of substances into and out of it as a result of aerobic respiration.

Notes:

Session:

Root structure

- ▼ Roots provide anchorage, and take up water and inorganic ions from the soil solution.
- ▼ As roots have to resist predominately pulling strains, the vascular (transport) tissue containing the phloem and woody xylem is found in the centre.
- ▼ Uptake of water and ions from the soil solution is via the root hairs which present a large total surface area for uptake.
- ▼ Root hairs are found just behind the actively growing root tips of young roots.
- ▼ The endodermis is a cylinder surrounding the central vascular tissue, it is composed of cells the

▼ The xylem tissue transports water and ions up to the stem and leaves, and the phloem transports organic substances especially sucrose from the leaves to the roots.

Notes:

Write a paragraph on how roots are adapted to their function.

Physiology and Transport

3.4 The transport of substances in Plants

Uptake and the transpiration stream

Check List

- ▼ The uptake of water from the soil is by osmosis when it is actually entering and passing through living cells (symplast pathway), and cohesive pull of the transpiration stream when it is passing through the porous cellulose cell walls directly to the xylem (apoplast pathway).
- ▼ The uptake of ions is by diffusion, facilitated diffusion and active uptake, and they may be swept up by solvent drag via the apoplast pathway.
- ▼ In all cases the passage cells of the endodermis exert some control.
- ▼ The active accumulation of ions within the barrier formed by the endodermis followed by the passive uptake of water by osmosis, results in the development of a root pressure which can force water up the xylem for a limited distance.
- ▼ Transpiration is the loss of water vapour from the leaves.
- ▼ It is an unavoidable consequence of having stomata opening for the uptake of carbon dioxide (and release of oxygen) in the light.
- ▼ Evaporation provides upward movement of a water column held together by cohesion (cohesion-tension) between water molecules in the xylem - the transpiration stream.
- ▼ The transpiration stream provides for upward transport of water and dissolved inorganic ions (and some organic compounds) from roots to leaves.
- ▼ Evaporation from leaves provides some cooling effect, but not when most needed as stomata shut and leaves wilt in conditions of extreme heat.
- ▼ Transpiration rate is affected by any factor that affects evaporation of water: relative humidity, temperature, air speed, availability of water, light intensity, degree of opening of stomata, number and distribution of stomata.
- ▼ Anything that increases the water potential gradient between the interior of the leaf and the external air increases transpiration.

Student Activity

Materials

- Various non-'hairy' leaves
- Clear nail varnish
- Microscopes
- Slides of TS leaf showing stomata
- Glass slides and coverslips
- Small 'pots' to stand on an electronic balance.

Procedure

Paint the various surfaces of the leaves with clear nail varnish, allow to dry, peel off dry film, and mount in water on a clean slide under a cover slip.

Observe impressions of stomata and count/estimate the number visible on a variety of leaves under $\times 100$.

Observe the TS of the leaf for views of the stomata in section.

Place a pot of water on a digital balance and record the loss of mass by evaporation under normal room conditions.

Repeat with fan and/or hair dryer with warm air, and try to record any differences in the rate of water loss by evaporation.

Set work

Write a paragraph on your observations of the distribution of stomata on leaves.

Notes:

Physiology and Transport

3.4 The transport of substances in Plants

Uptake and the transpiration stream

Topic Guide

Session:

Check List

- ▼ Transpiration can be measured using a potometer. The principle of which, is that as the water evaporates from the leaves of the shoot, water is moved up the shoot by the cohesive pull of the transpiration stream, and an air (bubble) is drawn along the capillary tube at the same rate - thus giving a measure of the rate of transpiration. The time taken for the air bubble to travel a certain distance, or the distance travelled in a certain time, can be recorded and the rate of transpiration calculated.
- ▼ Actually it is the rate of water uptake that is being measured, which under these experimental conditions is not the same as the transpiration rate. In this experiment the cross sectional area of the cut shoot is not as large as the equivalent area of root hairs that would normally supply the leaves, therefore the rate of uptake is less than the rate of transpiration.
- ▼ The potometer is filled by immersing it totally in water, preferably freshly boiled and cooled water which has no air bubbles.
- ▼ The shoot to be used should be cut from the plant and fitted into the potometer under water, to prevent any air bubbles entering the xylem elements.
- ▼ All joints should be well vaselined to seal leaks
- ▼ If the calibration on the capillary tube is in millimetres, and the diameter and hence the radius of the tube is known, then the volume of water taken up can be calculated using the formula:
$$\pi r^2 \times \text{distance travelled by bubble} = \text{volume of water uptake}$$
- ▼ Sometimes the calibrations are already in units of volume, for example mm^3 or cm^3 (ml). After the bubble has travelled a certain distance, it is returned to the beaker end by running water in from the reservoir.

Student Activity

Materials

- Practical Work Sheet:
Factors affecting the rate of transpiration using the potometer.
- Supplementary Practical Sheet:
Water loss from leaves

Procedure

Appreciate that although transpiration causes water to rise up the stem to replace that which is lost, a measurement of transpiration is not a measurement of water uptake from the soil.

The assumption is that the measurement of distance travelled by the air bubble in a given time is directly equivalent to the rate of transpiration.

List other factors which affect the rate of water uptake from the soil e.g the active uptake of inorganic ions by root hair cells giving rise to an osmotic gradient.

List the variables which might affect the rate of transpiration: temperature, humidity, air movement,

light. What is the relationship between light and stomatal opening?

Set work

List the sources of error when using the potometer, and in each case explain how the results would be affected.

Display your results and discuss problems encountered.

Check that the graphs show the rate of transpiration on the y axis and any variable factor on the x axis.

Notes:

Topic Guide

Session:

Physiology and Transport

3.4 The transport of substances in Plants

Xerophytes

Check List

- ▼ Thickened waterproof cuticle reduces water loss through the surface of the leaf, especially on the upper surface of the leaf which is most exposed to air currents and heat absorption from sunlight.
- ▼ Surface of the leaf may be 'hairy' (not true hairs) which protect the stomata from air currents.
- ▼ Stomata can be sunken in pits and grooves, which protects them from air currents, e.g. on pine 'needles'.
- ▼ Stomata can be reduced in number, especially on the upper surface of the leaves, where the exposure to the heat of the sun and air currents is the greatest, indeed some e.g. laurel, have no stomata on the upper surface.
- ▼ Leaves can have a reduced surface area, which also reduces the number of stomata, e.g. pine needles'; and gorse and cacti where the leaves are reduced to spines.
- ▼ Leaf folding or rolling reduces water loss through the stomata by protecting them from air currents, and enclosing them in a zone of high humidity, e.g. *Ammophila* (sand dune grass).
- ▼ Leaves can be succulent, with tissues where water can be stored for periods of drought, e.g. *Mesembryanthemum*.
- ▼ Cells of the leaves have mucilage and can withstand dehydration for longer periods than those of non-xerophytes (mesophytes).
- ▼ Leaf fall stops transpiration. Deciduous plants lose their leaves with the onset of winter in temperate regions, when the low temperatures inhibit water uptake by the roots.

Student Activity

Materials

- Assorted plants and parts of plants showing a range of xerophytic adaptations e.g. cactus with spines, succulent with fleshy leaves, pine needles, holly leaves, marram grass, plus one mesophyte e.g. *Pelargonium*.
- Microscopes and slides
- Two leaves per student (one xerophyte e.g. holly and one mesophyte, e.g. *Pelargonium*) coated on their under surface with a layer of clear nail varnish.

Procedure

Compare the numbers and distribution of stomata on a xerophytic leaf e.g. holly and a mesophytic leaf. Carefully peel off the layer of nail varnish that has been painted on to the lower leaf surfaces of the two plant types and mount a small piece on a microscope slide. Examine the imprint of the epidermal surface and identify the guard cells and stomata. Count how many stomata occur in the field of view, then repeat this count on different parts of the slide. Record the average, then repeat using the other leaf (on the same

power of magnification). Compare the numbers of stomata on the two leaves.

Note that not all of the xerophytic adaptations are limited to plants living in hot dry climates. Other important groups of plants show xerophytic (water stress) modifications e.g.

Plants which shed their leaves all year round (evergreens) and which must survive long frozen winters (e.g. conifers)

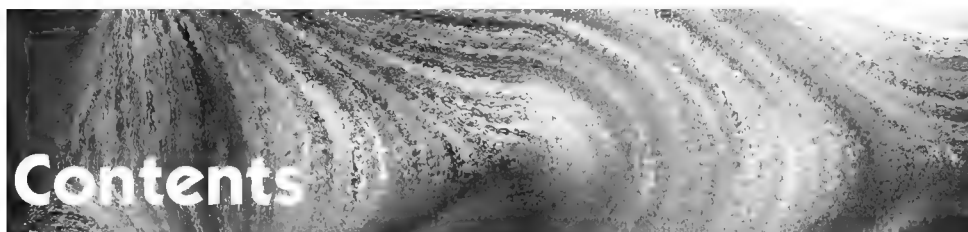
Plants adapted to salty environments (halophytes) e.g. salt marsh plants

Wet acid heath plants where water uptake is restricted by xerophytic modifications to avoid toxic levels of iron uptake.

Set work

Tabulate your results of stomatal counts and draw conclusions on the distribution of stomata in relation to water loss.

Practical Guide



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Practical Work Guide

An Introduction

This guide is designed to provide material for supervisors to use at their discretion.

All of the exercises provide opportunities to contribute to fulfilling the Aim that:

Biology should encourage students to:
develop an understanding of scientific methods.

Equally all of the exercises provide opportunities to contribute to:

devise and plan experimental and investigative activities, selecting appropriate techniques;
demonstrate safe and skillful practical techniques;
make observation and measurements with appropriate precision and record these methodically;
interpret, explain, evaluate and communicate the results of their experimental and investigative activities clearly and logically using biological knowledge and understanding and using appropriate specialist vocabulary.

They provide the opportunity for the assessment of:

- A. Planning
- B. Implementing
- C. Analysing evidence and drawing conclusions
- D. Evaluating evidence and procedures

The responsibility for safe and correct practice rests entirely with the designated supervisor, to whom the methods are offered as a source of information only.

Hazard Cards as produced by, for example CLEAPS, should be displayed wherever necessary.

CLEAPS, School Science Service, Brunel University, Uxbridge, UB8 3PH

Biology Core Principles

1.1 Biological Molecules

Biochemical tests

The identification of reducing and non-reducing sugars, starch, proteins and lipids by means of simple biochemical tests, using Benedict's solution, iodine solution, the biuret test and the emulsion test.

Simple biochemical tests

Introduction

A standard series of procedures are used to test for a variety of biological molecules. These tests are traditionally known as 'food tests', but here the basic test methods are performed on a standard solution of each type of substance.

Method - Benedict's test for reducing sugars

- ▼ Put on a pair of safety glasses. Collect a 500cm³ beaker and fill it about half full with tap water. Place it on a tripod and gauze over a Bunsen burner and bring the water to the boil.
- ▼ Place a small quantity of reducing sugar solution (about 1-2 cm³) in a boiling tube. Add 2cm³ of Benedict's reagent.
- ▼ Place the boiling tube in the waterbath and boil the tube and its contents for 3-4 minutes. Remove the tube from the waterbath using test-tube tongs and place it in a test-tube rack.

If the sample contains reducing sugars, a precipitate will be produced, and the colour will change from blue through green and yellow to brick-red, depending on the amount of sugar present. This test can thus be used to give an estimation of the amount of sugar present, provided the quantities of sugar solution and Benedict's reagent used are the same each time. A small amount of sugar will turn the solution green, larger amounts yellow and considerable quantities will turn it brick-red.

continued

Method - Benedict's test for non-reducing sugars

This test should be done if the previous test for reducing sugars gives a negative result.

- ▼ Place a fresh sample of sugar solution about (1-2 cm³) in a boiling tube and add 1cm³ dilute hydrochloric acid. Place the boiling tube in the waterbath and boil it for 1-2 minutes.
- ▼ Remove the test-tube from the waterbath using tongs and place it in a test-tube rack. Allow to cool for a couple of minutes. Using a spatula, add small quantities of sodium hydrogen carbonate powder, until the mixture stops fizzing. (This will probably take about 3 small spatulas full: the sodium hydrogen carbonate neutralises the acid)
- ▼ Add 2cm³ Benedict's reagent to the boiling tube and put it back in the waterbath, using the tongs. Boil it again for 3-4 minutes.

If non-reducing sugars (e.g. sucrose) were present in the original sample, the acid will hydrolyse them to monosaccharides, which are all reducing sugars: they will then give a green/yellow/brick-red precipitate with Benedict's reagent.

Method - Samples containing both reducing and non-reducing sugars

If you suspect that a sample contains both reducing and non-reducing sugars:

- ▼ Test with Benedict's reagent and obtain the precipitate as described above (using double quantities of sample and reagent)
- ▼ Filter the tested sample through filter paper, and collect the filtrate.
- ▼ Test the filtrate with Benedict's reagent.
- ▼ Continue this process, using the filtrate each time, until you no longer get a positive result with the Benedict's test.
- ▼ Add 1cm³ dilute hydrochloric acid to the remaining filtrate and boil it to hydrolyse any non-reducing sugars it contains. Neutralise the mixture and re-test with Benedict's reagent.
- ▼ If a non-reducing sugar was present in the original sample there will be a positive result once again.

Method - Iodine in Potassium Iodide Solution test for starch

- ▼ Place a small sample of starch solution on a spotting tile or in a test-tube.
- ▼ Add a few drops of iodine in potassium iodide solution.

The starch will combine with the iodine to give a deep blue-black colour. This test is very sensitive, so only tiny quantities are needed. The colour will fade after a while.

continued

Method - Biuret test for proteins

- ▼ Place 2 cm³ of protein solution in a test-tube. Add 2 cm³ of dilute potassium hydroxide solution, then add 0.5 cm³ of dilute copper sulphate solution, a drop at a time. Shake the test-tube gently, then leave it in the test-tube rack for a few minutes.

If protein is present, a purple colour will develop after a few seconds (s). If there is no protein, the solutions will remain very pale blue from the copper sulphate solution.

Method - Emulsion test for lipids

This test uses the fact that alcohols will dissolve lipids, but water will not.

- ▼ Place a small sample of lipid in a test-tube. Add 1-2 cm³ of alcohol and shake the test-tube thoroughly for a few minutes. Place it in a test-tube rack and allow it to settle.
- ▼ Collect a clean test-tube and half fill it with tap water. Carefully pour the alcohol layer from the sample into the water.

The lipids will dissolve in the alcohol as the sample is shaken. When the solution is poured into the water, the alcohol will dissolve in the water, but the lipids will come out of solution and form fine droplets. Thus if there are lipids in the sample, they will form a cloudy white emulsion in the water.

These methods may be used for testing materials in a laboratory, or for testing foodstuffs to analyse their contents.

Teaching Notes

For the emulsion test, either ethanol or iso-propanol will work, provided it is a strong solution (at least 70%).

The only problem with the standard tests is doing the emulsion test on milk, in which the emulsion effect is masked. The Sudan III test for lipids may be more appropriate for this situation, as it gives a clearer result. If milk is shaken up with Sudan III and methylene blue and allowed to settle, the red Sudan III will separate out in the fatty layer at the top, and the methylene blue in the aqueous layer at the bottom.

The test for reducing sugars can be made quantitative by carrying out the Benedict's test on a series of known concentrations of glucose solution, to produce a colour series, then testing some unknowns to identify where they fit in the series. This could be introduced as a problem for the students, to provide experience in planning experiments.

Clinistix or equivalent can be used to test for different glucose concentrations.

This is a standard practical. You could try varying it, for example, by suggesting that they are testing foodstuffs for a supermarket firm who have been told that some of their foods have been spiked: try adding starch to the milk, or injecting some fats into oranges, for example.

Alternatively, there is a good variation published in Journal of Biological Education, (Roger Law, 1995, "An A-level food test investigation", JBE, Volume 29, p251-252), which uses other tests as well, to analyse mock "stomach contents" from two animals. The idea is to decide whether they are carnivorous or herbivorous. This test uses some solutions which are a bit more risky than the usual ones (Schultz' reagent for cellulose, for example.) However, it can make an enjoyable change.

Requirements per student

Boiling tubes
Test tubes
Small measuring cylinders or dropping pipettes
500cm³ beaker
Bunsen burner, tripod and gauze
Test-tube rack
Test-tube tongs
Spotting tile
Small spatula
Filter funnel and filter paper
Safety glasses

Benedict's reagent
Dilute hydrochloric acid
Sodium hydrogen carbonate powder
Iodine in potassium iodide solution
70-80% alcohol solution
Biuret 1% copper sulphate solution
Biuret 15% potassium hydroxide solution

Standard solutions of sucrose (5%), glucose (5%), Starch (1%), albumin (5%) and a small sample of cooking oil.

continued

Safety Considerations

The following chemicals need to be handled carefully:

Dilute hydrochloric acid – corrosive

Iodine in potassium iodide solution – irritant

80% alcohol – highly flammable

Biuret potassium hydroxide – corrosive

Safety glasses should be worn for the Benedict's test at least: you may prefer students to wear them all the time.

The Benedict's reaction should be boiled in a waterbath – never allow students to heat the tube directly in the Bunsen flame.

Normal precautions for handling hot apparatus should be observed.

Biology Core Principles

1.1 Biological Molecules

Chromatography

The separation and identification of compounds by means of chromatography, including R_f value and two way chromatography.

Chromatography

Introduction

Chromatography is a technique used to separate and analyse which molecules are present in a mixed extract from living material. It depends on the use of organic solvents, which dissolve the molecules concerned. These solutions are allowed to move up a thin layer of inert material, such as paper or a thin layer of silica gel, carrying the molecules with them. The distance the molecules move is related to the size and shape of the molecule, as well as their relative solubility in the solvent and the water associated with the layer of inert material.

For each combination of molecule and solvent, the distance moved relative to the solvent alone is characteristic, and can be used to identify the molecule. The value is known as R_f (Relative front) and is calculated thus:

$$R_f = \frac{\text{Distance moved by molecule}}{\text{Distance moved by solvent}}$$

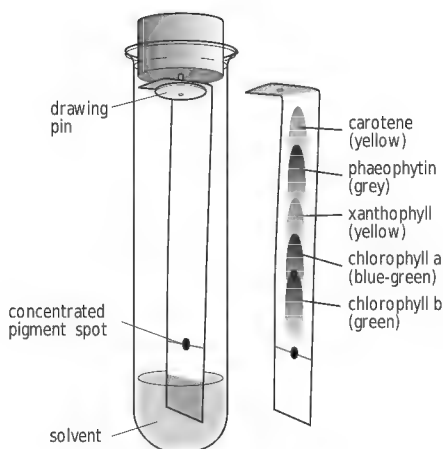
Method

- ▼ Cut a piece of chromatography paper (or filter paper) about 1.5cm wide, and long enough to reach the bottom of the boiling tube. Fold the top 1cm over, and make sure the paper will fit into the boiling tube, with the folded edge at the top, the end nearly reaching the bottom, and the sides NOT touching the sides of the tube. If necessary, trim it to fit.
- ▼ Rule a pencil line (not pen!) across the paper about 2.5 cm from the bottom. Mark the centre of the line with a cross. Write your name at the top of the strip in pencil.
- ▼ Collect a handful of plant material, and cut it up quite finely with a sharp knife. Place 5 cm³ of propanone in the mortar, and grind up the plant material in the mortar, using the pestle and a small amount of sand, (or use a liquidiser). The green pigments should dissolve into the propanone. As you grind up the plant material, remove any tissue that is well ground, and add more, but do not add any more propanone: you need to get as concentrated a solution as possible.
- ▼ Once you have a concentrated solution, filter the contents of the mortar through a piece of gauze in a filter funnel. Discard the solid materials.
- ▼ Put a small amount of solvent (to a depth of not more than 1.5 cm) in the bottom of the boiling tube. Put the bung in the tube and leave it on one side so that the air in the tube can saturate with the solvent vapour.

continued....

- ▼ Using a piece of very fine capillary tubing or the head end of a pin, place a drop of the extract on the cross in the middle of the pencil line of the paper strip. Use a hair dryer to dry the drop, then put another drop in the same place. Repeat this process until you have a small area of concentrated pigment. Be patient with this: you will get a much better result if you let each drop dry properly before you add the next. You should add at least 5-6 drops.
- ▼ Use a drawing pin stuck into the base of the bung, or a split bung, to suspend the paper in the boiling tube, so that the end of the paper reaches into the solvent, but the spot of pigments is not in the solvent. Adjust the amount of solvent if necessary.
- ▼ Leave the boiling tube in a rack to allow the solvent to move up the filter paper. When the solvent has nearly reached the top of the strip of paper, remove the bung and detach the paper. Draw a pencil line on the paper to mark the level reached by the solvent, then leave it on the bench (or in a fume cupboard) to dry.
- ▼ Measure the solvent distance, and the distances moved by the spots of pigment. Identify the pigments by their colour and R_f values, using the information given in the table below. If you have produced a good concentration of pigments in the extract and spot, you should be able to identify all five pigments.

Pigment Name	R_f value	Colour
Carotene	0.95	Yellow
Phaeophytin	0.83	Yellow-Grey
Xanthophyll	0.71	Yellow-Brown
Chlorophyll a	0.65	Blue-Green
Chlorophyll b	0.45	Green

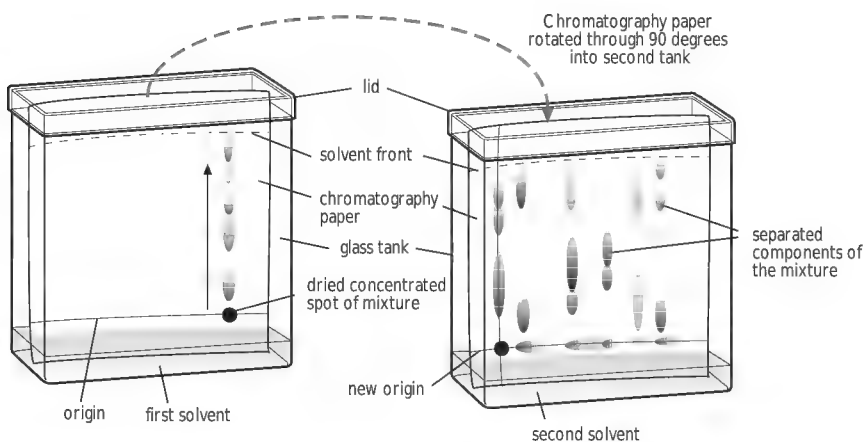


continued

Method - Two way chromatography

Two way chromatography is used to make further separations of substances that may not have been separated sufficiently on the first run of a chromatography investigation.

The chromatography paper from the first run is turned by 90° and re-run as shown in the diagram. Details are provided with Chromatography kits, and are not repeated here as no new principles are involved.



Teaching Notes

The solvent is made from 1 part 90% Propanone (Acetone) to 9 parts Petroleum ether (alternatively 12 parts: 100 parts)

The plant material needs to be as green as possible! Nettle leaves work well, and can be handled easily with gloves. Alternatively, spinach leaves also give a good result. As a possible extension, or for a student project, it might be interesting to try a different coloured leaf, such as red cabbage, although the R_f values for any extra pigments may be difficult to find.

One important factor in getting the practical to work well is to get the chlorophyll extract as concentrated as possible: the students need to spend time over this, and grind up quite a lot of plant material in as little propanone as they can. The other stage at which patience is needed is the preparation of the pigment spot. They must let each drop dry thoroughly, before adding the next, and they need to add at least 5-6 drops. Using a hair dryer speeds up the process. To keep the evidence of the experiment; after allowing the filter paper to dry, it should be sealed under Sellotape so the pigments do not fade.

If you have access to thin layer plates, they tend to work better than paper sheets, but are more complex to set up. If you do use thin layer plates, the R_f values will be different from those given here.

Requirements per student

- Filter or chromatography paper
- Scissors
- Boiling tube with a rubber bung
- Drawing pin
- Ordinary pin or piece of fine capillary tubing (especially fine chromatography droppers are available)
- Pestle and mortar or liquidiser
- Sand for grinding
- Pencil and ruler
- Sharp knife
- Cutting tile
- Filter funnel
- Piece of gauze, about 12 cm square
- 50 cm³ beaker
- Test-tube rack
- 5 cm³ propanone
- 10 cm³ solvent
- Plant material, e.g. nettles, spinach etc.

Safety Considerations

Both the propanone and the solvent are highly flammable and need to be handled with care. The lesson should be run in a well-ventilated room, and all naked flames should be extinguished. Ideally the strips should be dried in a fume cupboard.

If you use nettle leaves, they should be handled wearing disposable rubber gloves of the surgical type or ordinary rubber gloves, these will be better than the very thin film gloves. Once crushed or cut up, the nettles can be handled with bare hands.

Biology Core Principles

1.2 Cells

Cell structure

The structure of an epithelium cell from the small intestine and a palisade mesophyll cell from a plant as seen with a light microscope.

The observation of animal and plant cells under the light microscope

Introduction

A prepared slide of the mammalian small intestine in cross or transverse section (T.S.) is to be observed for the identification of the lining epithelium tissue.

The epithelial cells are column shaped, with an oval, darkly stained nucleus towards the bottom of the cell. The free edge of the cell has a 'brush' border of microvilli - but this will usually only be seen as a narrow 'different looking' layer at the top of the cell, where it faces into the lumen (space) of the gut. Goblet cell secreting mucus are also present, and quite distinctive.

Palisade mesophyll cells will be observed in a prepared slide of a leaf cut in cross section (T.S.). The palisade mesophyll cells form a layer (or layers) of cells below the upper epidermis of the leaf. They are oblong in shape, but may be quite badly disrupted in the preparation of the slide. Even under a high power (x40) objective lens it may be difficult to see cell structures, except for cell walls and chloroplasts.

Method

- ▼ Examine the prepared slide of T.S. leaf first. Locate the palisade cells using the medium power (x10) objective lens, you will probably need a high power (x40) objective lens to do the drawing.
- ▼ Draw 2-3 adjacent palisade cells (at least a few cm across), and label the structures you can identify. This may only be the cell walls, and the chloroplasts which are pushed up against them. Annotate the labels to indicate the functions of the parts you have drawn.
- ▼ Calculate the magnification of the drawing thus:

Magnification by microscope = Eyepiece lens magnification x objective lens magnification

Record this figure. Now correct the magnification for the size of the drawing, that is the number of times the drawing is larger than the image seen down the microscope, e.g. twice the size (x2).

This gives a final magnification of:

Eyepiece lens x objective lens x 2

Also record this figure.

continued . . .

It is possible to measure the actual size of the specimen.

See Supplementary Practical Sheet: 'Measuring specimens under the light microscope'

- ▼ Examine the prepared slide of T.S. small intestine under the medium power ($\times 10$) objective lens. The epithelial cells are much smaller than plant cells, and are much more indistinct as they lack the cellulose cell wall found in plant cells. You will need to move onto a high power ($\times 40$) objective lens once you have located the column shaped cells on the inner edge of the intestine wall.
- ▼ Draw 2-3 cells, enlarging them as much as you can. Label the structures you can identify and annotate the labels.
- ▼ Calculate the final magnification of the drawing and record it.
- ▼ Make sure both drawings have a suitable heading.

Teaching Notes

With plant tissues the student can usually see the cell walls and chloroplasts, the vacuole and nucleus. However, the cells can be quite badly distorted, and poorly stained, and therefore difficult to draw. It might be a good idea to get them to draw xylem elements in T.S. to gain some experience of drawing plant cells.

In the much smaller epithelial cells the nuclei should be seen, but they often overlap each other. The microvilli appear as a narrow layer along the free edge of the cells.

(It is interesting to note that many organelles were first described using the light microscope (some over 100 years ago) e.g. Golgi body (apparatus), mitochondria, and endoplasmic reticulum).

This practical also provides an opportunity to discuss the main points of **biological drawing technique**.

The important points to get across are:

- ▼ Drawings should be line drawings, with no shading, or use of colour.
- ▼ They should be large enough to be clear.
- ▼ The lines should not be sketchy.
- ▼ Pencils should be sharp, HB or H.
- ▼ Label lines should be drawn with a ruler, and should not cross. The labels should be written in pencil, and should be well away from the drawing.
- ▼ Labels should be annotated, either next to the label or as a suitable note, to give further information as required.
- ▼ Magnification, or an indication of size/scale, should be included.
- ▼ Drawings should be clearly labelled with the student's name, the name of the specimen, its classification if appropriate, type of section / squash etc., and preparation or staining details.
- ▼ Sometimes, depending on the microscope and the slide, the plant cell wall cannot be clearly seen to have a thickness, but it is always drawn with a double line to distinguish it from membranes.

Requirements per student

Microscope

Drawing materials

Prepared slides of T.S. mammalian small intestine

Prepared slides of T.S. leaf

Supplementary Practical Sheet: Measuring Specimens under the Light Microscope

Safety Considerations

There are no special safety considerations for this practical other than the safe use of the microscope.

Biology Core Principles

1.2 Cells

Cell structure

The structure of an epithelium cell from the small intestine and a palisade mesophyll cell from a plant as seen with a light microscope.

The estimation of size of microscopic structures

Introduction

There are two methods you can use to measure the size of objects under the light microscope. One is a calculation based on the magnification, the other is to use an eyepiece graticule, which will need to be calibrated for the microscope using a stage micrometer. The graticule will give you a more accurate measurement, but the procedure is more complex.

Method A - Using the magnification

Make a note of the magnification of the eyepiece lens, and of the objective lens. The total magnification is found thus:

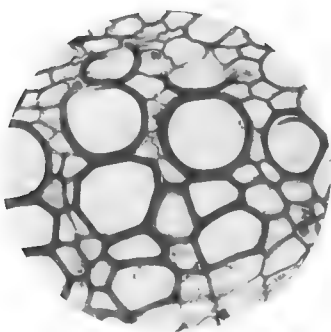
Total magnification = magnification of eyepiece lens x magnification of objective lens

The term **linear magnification** refers to the number of times an imaginary 'line' across the drawing is greater than the equivalent would be on the original specimen (as opposed, for example, to considering the magnification of the area).

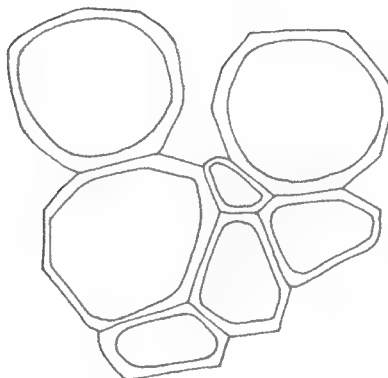
To calculate the linear magnification of the drawing, estimate how large the drawing is, compared with the view down the microscope. Look down the microscope, and then at the drawing, is the drawing x2, x3, x4 the size of the specimen?

You can then multiply the magnification by the size difference.

So if this is what you can see:
(with x100 magnification)



and this is what you have drawn:



If the eyepiece lens is x10, and the objective lens is x10, then:

Final magnification = $10 \times 10 \times 2$ (drawing is about twice the size of the specimen) = x200 magnification

To work out the size of the original object under the microscope, measure a line across the drawing in mm, and divide it by the total magnification, giving the answer in μm .

Method - Using the eyepiece graticule

- ▼ To do this, you need to have a scale put in the eyepiece lens, so that it can be seen when you look down the microscope. These scales are usually on small circular pieces of glass or acetate.
- ▼ To insert the scale in the eyepiece, remove the eyepiece lens from the microscope, and carefully unscrew the top lens. If you look down into the lens body, you should see a ledge running round the sides about halfway down. Drop the scale into the lens body, so that it rests on the ledge, then replace the top lens. It does not matter if the scale is the wrong way up, but if it annoys you, unscrew the lens again and turn the scale over.
- ▼ When you look through the microscope, you should see the scale overlying the specimen.
- ▼ To calibrate the scale, you need to use a stage micrometer. This can be a special slide with a scale engraved on it, or one can be made by mounting a scale on a slide under a coverslip using Canada Balsam. It usually consists of a scale 1cm long, which is divided into 100 units, each of which is 0.1 mm (100 μ m), there is an extended line every 10th unit. It is worth being aware that these scales can be badly scratched.

To calibrate the eyepiece scale

- ▼ Place the stage micrometer on the microscope stage and hold it down with the clips.
- ▼ Using the eyepiece lens with the scale in, look through the microscope and focus it so you can see both scales clearly. This is usually easier if you focus the eye on the eyepiece scale and adjust the microscope so the stage scale comes into focus as well.
- ▼ Move the stage micrometer carefully so that the starting units of the two scales coincide.
- ▼ Count along the two scales until there is again a coincidence between the two scales. Note down the number of divisions along each of the two scales that this represents.
- ▼ As each division along the stage micrometer scale represents 100 μ m, then:
$$\begin{array}{l} \text{1 division on the eyepiece scale} \\ \text{(in mm)} \end{array} = \frac{\text{Number of divisions on the stage micrometer scale}}{\text{Number of divisions on the eyepiece graticule scale}} \times 10$$
- ▼ At $\times 100$ and $\times 400$ magnification the lines on the scale will have a definite thickness. It is important to measure from one side of one scale mark, to the same side of the next coinciding mark.
- ▼ This procedure should be carried out for all the objective lenses on the microscope, so that you have a scale calibration for all possible magnifications. Record the information in a table with the following headings.

Power of lens	Magnification	Eyepiece scale 1 division (in mm)

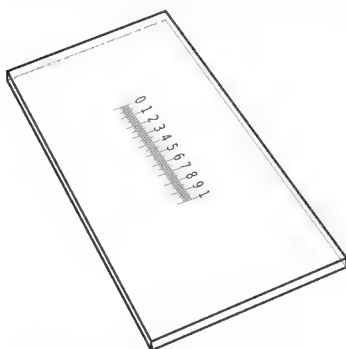
- ▼ When you measure another specimen, you will already have the calibration figures, so all you have to do is count how many eyepiece scale divisions the specimen covers, and multiply that by the calibration factor for that objective lens.

Measurement of size under the microscope

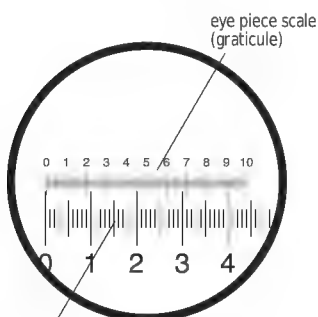
Eye piece scale (graticule)



Eye piece scale as seen when held up to the light away from the microscope.

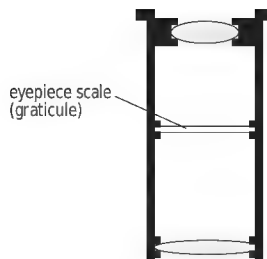


Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



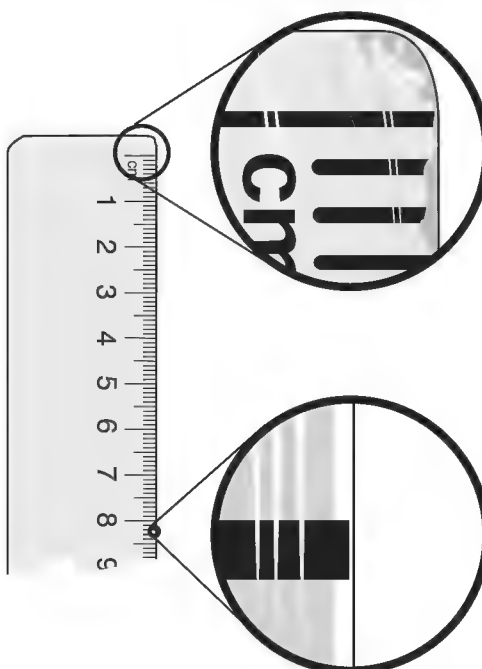
slide micrometer scale

Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.



eye piece scale (graticule)

More of the object is seen under low power objective lens



Less of the object is seen under high power objective lens

Teaching Notes

Individual help is often required to ensure that students fully understand the procedure of using the eye piece graticule.

The plastic ruler analogy may help, and by giving an estimate of the diameter of the field of view can actually provide a crude measure of size of microscopic structures seen under the light microscope.

Requirements per student

Microscope

Eyepiece graticule

Slide micrometer

Suitable prepared slides

Supplementary Practical Sheet: Measuring specimens under the light microscope

Reference books / pictures / class notes on plant and animal histology

Safety Considerations

There are no special safety considerations with this practical other than the safe use of the microscope.

Biology Core Principles

1.3 Cell transport

Water potential

Osmosis as a special case of diffusion across a partially permeable membrane, net movement of water depending on difference in water potentials.

Hypotonic, hypertonic and isotonic solutions, and the importance of ion concentrations in maintaining cell turgor

An investigation into the water potential of plant cells

Introduction

In this practical the changes in mass caused by water loss or gain due to osmosis are to be investigated. Pieces of plant storage tissue, e.g. potato, are soaked in distilled water and sucrose solutions of different concentration. The changes in mass of the tissue reflect changes at the cellular level.

Method

- ▼ Collect a set of six 50cm³ beakers, and label them 0%, 0.5%, 1%, 5%, 10%, and 15%. Put 30 cm³ of distilled water in the 0% beaker.
- ▼ Using the 15% stock solution of sucrose supplied, make up the dilutions of sucrose solution listed, and put 30cm³ of each dilution in the appropriate labelled beakers.
- ▼ Collect a potato and use the cork borer to cut out a cylinder of potato tissue. Carefully cut the cylinder into discs 1mm thick, discarding the ends with the skin on. You will need about 60 discs.
- ▼ Using a balance, weigh 10 of the potato discs, note the mass and record in a suitable table. Repeat 5 times, so you have 10 discs of known combined mass for each solution.
- ▼ Add 10 discs of known combined mass to each of the solutions. Note the time.
- ▼ After 45-60 minutes, remove each set of discs from the beakers, very carefully blot them dry on a paper towel or tissue and reweigh them. Make a note of the new masses in the table.
- ▼ Calculate the % change in mass of the potato tissue in each liquid by dividing the gain or loss in mass by the original mass and multiplying by 100. Plot a graph of % change in mass against concentration of sucrose solution (include you distilled water result).
- ▼ Note the turgidity of the discs in the different solutions.
- ▼ Turgidity may be quantified by repeating the experiment with 'chip' shaped pieces of potato, pushing a long pin into the end of each, and pinning the strips onto a cork board covered by graph paper and measuring the degree of 'droop'.

Discuss what the results indicate about osmosis and the effect that it has on living tissue.

Use the graph to estimate which concentration of sucrose solution possibly matches the internal concentration of the potato cells (isotonic).

Teaching Notes

This practical works well, and is better than the standard potato chip exercise, as the surface area / volume ratio is higher and the osmotic exchanges proportionally greater.

The discs are fairly easy to cut, but to save time, the discs can be cut beforehand and kept in 5% sucrose solution.

Some students may need help calculating the volumes of sucrose solution and distilled water to get the correct dilutions.

If possible you need a balance which will show 2 decimal places to get an accurate result.

Students must be very gentle when blotting discs before reweighing

With regard to measuring changes in mass as opposed to changes in length of chip, or diameter of onion ring, the problems associated with blotting off excess fluid must be considered. Even when blotted with care, much fluid remains associated with the cellulose cell walls, which will have less effect on the linear dimensions than it will on mass.

(Changes in onion ring diameter in different concentrations of solution/changes in mass of slices of banana of different ripeness/comparing change in the mass of slices of different plant materials e.g. carrot, parsnip, swede etc. are all worth a try).

Requirements per student

Cutting tile
1.5 – 2 cm diameter cork borer
Sharp knife
6 x 50cm³ beakers
50cm³ measuring cylinder
Syringes/pipettes
Balance
Paper towels/tissues
Pins
Cork board
Graph paper

100 cm³ 15% sucrose solution
Distilled water
Potato

Safety Considerations

The students need to be careful with the cork borer and sharp knife while preparing the potato discs.

Biology Core Principles

1.5 Enzymes

Enzyme properties

The effects of change in temperature, pH, substrate concentration, and competitive and non-competitive inhibition on the rate of enzyme action

An experiment to investigate the effect of temperature on the activity of Catalase

Introduction

Catalase catalyses the breakdown of hydrogen peroxide to oxygen and water.



The rate of production of oxygen can be measured, and gives a measure of the rate of the reaction.

Catalase is produced in cells to prevent the accumulation of hydrogen peroxide, which is toxic.

In this experiment potato tissue is used as a source of catalase.

The effect of temperature on the rate of this reaction will also be investigated.

TAKE CARE with the HYDROGEN PEROXIDE - wear safety glasses and try not to get any on the skin, wash it off immediately if you do.

Method

▼ There are various pieces of apparatus that you can use to collect and then measure the volume of oxygen gas that will be evolved when the catalase in the potato tissue reacts with the hydrogen peroxide.

a The oxygen gas can be collected over water in an inverted measuring cylinder.

b The oxygen can be collected over water in the inverted barrel of a 20 cm³ syringe.

c The oxygen can be collected and measured in a manometer tube filled with coloured fluid, linked to the reaction vessel. You will advised which method(s) to use.

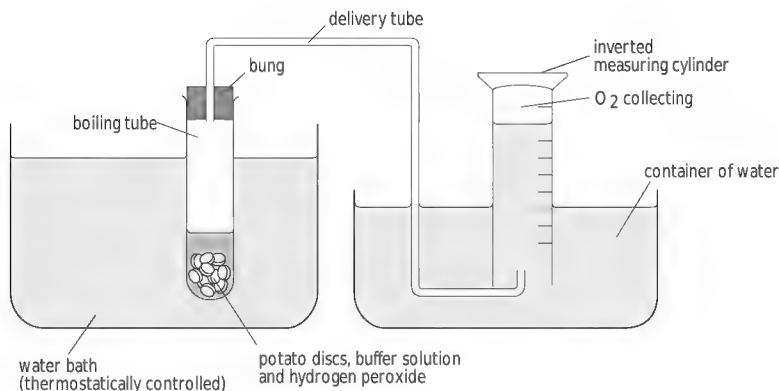
The details below relate mainly to the 'inverted measuring cylinder' method.

▼ Using a cork borer a little smaller in diameter than the boiling tubes, cut some long potato cylinders. Then using a knife (scalpel) carefully cut the potato cylinder into discs about 1 mm thick.

▼ Place the discs into a few cm³ of pH7 buffer in a watch glass to keep them moist. You will need 50-60 discs.

▼ Using a boiling tube as the reaction vessel, place about 10 potato discs into the tube and just cover them with a measured volume of pH7 buffer.

continued



- ▼ Place the tube in a water bath, at the first temperature you intend to use, e.g. 20°C and allow the discs time to reach the correct temperature. Place a boiling tube of hydrogen peroxide into the water bath at the same time, so it also reaches the same temperature.
- ▼ Assemble the apparatus; using a rubber bung with a delivery tube inserted, and check that this will fit under the inverted 25 cm³ measuring cylinder full of water in its container of water. You may have to use a clamp stand to support the cylinder.
- ▼ When you are ready, measure 5cm³ of hydrogen peroxide (H₂O₂) in a syringe. Carefully loosen the bung of the boiling tube, and very quickly and safely add the peroxide. Replace the bung and start the stopclock, tap or shake the tube if necessary to mix the contents.
- ▼ Record the volume of oxygen produced every 30 s for 3 minutes.
- ▼ Remove the boiling tube, wash it out and add 10 new discs and the same volume of pH7 buffer as used before. Put the tube back in the water bath and carefully raise the temperature by 10°C.
- ▼ If necessary refill the measuring cylinder with water.
- ▼ Add 5cm³ hydrogen peroxide to the boiling tube, quickly replace the bung, start the stopclock and take the readings as before.
- ▼ Repeat the experiment at 30, 40, 50 and 60°C using a new set of potato discs each time.

Look at the rate at which the oxygen has been produced during each 30s period. Can you explain the results in terms of an enzyme reacting with its substrate?

Calculate the mean amount of O₂ produced during the 3 minute period, at each temperature. Plot a graph of these figures against temperature.

How could you improve the method to obtain more reliable results.

Plan how you could adapt this experiment to investigate the effect of pH, substrate concentration, or enzyme concentration, on the action of Catalase. Check the new plan with the supervisor, then carry out the investigation and write a full report, including analysis of results, conclusion, evaluation etc.

Teaching Notes

This practical usually works quite well and it is a good exercise for students to demonstrate their manipulative skills. A number of methods can be used in this experiment to measure the volume of oxygen produced, as mentioned on the student sheet: e.g. over in an inverted measuring cylinder, over water in the barrel of a syringe (a small piece of rubber tubing is attached to the narrow end of the syringe which has a screw clip so the barrel can be filled with water) or in any simple manometer.

A bit of juggling with the number of potato discs and the volume of peroxide (or its concentration) may be necessary to achieve a good result. But students using the inverted measuring cylinder method at 40°C recently obtained results of:

O₂ produced: 30s/5 cm³, 60s/2cm³, 90s/1.5cm³, 120s/1.0 cm³, 150s/0.5 cm³
180s/0.5 cm³.

Hydrogen peroxide can be added from a syringe, the needle of which is inserted through the cork, to improve accuracy.

If the students are going on to measure the effects of pH, they will need to be supplied with quantities of buffers: pHs suggested are 4, 5, 6, 7, 8, 9. They will need only a few cm³ of each buffer per student.

Substrate concentration can be varied using different concentrations of hydrogen peroxide, such as 5 vol., 10 vol., 15 vol., and 20 vol.

Effect of enzyme concentration can be measured by varying the number of potato discs used. Alternatively you could vary the size of the discs (either diameter or thickness). It is quite important that the discs are cut to the same sizes for this version of the experiment. This would also allow them to consider the effect of surface area: volume ratio.

The effect of competitive and non-competitive inhibition presents greater problems.

Non-competitive inhibition could be tried with amylase and starch. Iodine inhibits amylase and is part of the test for the disappearance of starch, so the investigation of the non-competitive inhibition of amylase by iodine is highly relevant.

Competitive-inhibition can be investigated using the respiratory enzyme succinic dehydrogenase and the two substrates succinate and malonate, but as the respiratory pathways are common to all living organisms, there is a risk factor involved in their use. None of the team have performed this experiment.

continued

Requirements per student (measuring cylinder method)

Boiling tube
Large beaker (500 cm³)
Bunsen burner
2 tripods and gauzes
or thermostatically controlled water bath and 1 tripod and gauze
Bung with delivery tube
Measuring cylinders 25 cm³ and 10 cm³
Trough or equivalent
Cork borer 10mm diameter (No6)
Watch glass
2 100cm³ beakers
5 cm³ syringe or dropping pipette
1 clamp stand
Stopclock
Thermometer

Knife/scalpel
50cm³ pH 7 buffer
50cm³ 20 volume hydrogen peroxide
Potato

Safety Considerations

Use of the sharp knife and cork borer need care.

Hydrogen peroxide is an oxidant, and needs to be kept away from heat and stored in a fireproof cabinet.

Hydrogen peroxide can also be an irritant or corrosive at strong concentrations, so care is needed especially with the 15/20 volume.

Safety glasses, overalls and disposable gloves should be worn and contact avoided.

Biology Core Principles

1.6 Digestion

Extracellular digestion

The principles of the use of starch agar plates for assaying carbohydrase activity

An investigation into the action of amylase on starch agar plates

Introduction

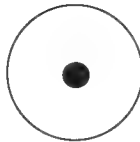
Agar plates containing starch are a simple means of estimating the activity of carbohydrase enzymes. The clear zones found after treatment with iodine in KI solution can be used to compare the concentrations (assaying) of amylase in various solutions. Alternatively, it may provide a means of investigating the action of amylases from different sources.

Method

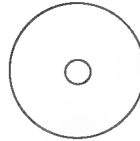
- ▼ Collect 7 petri dishes of starch agar, and label them with your name and the following concentrations of amylase solution: 5%, 2.5%, 1%, 0.1%, Boiled 5% amylase solution control. Also unknown(s), and methylene blue. Do not open the lids until you have to, and then try to work quickly, replacing the lids as soon as possible.
- ▼ Using a sterilised cork borer, make a well in the centre of each plate. Remove the plug of agar with a mounted needle if it does not come out with the cork borer. Put the plates on a side bench where they may be left undisturbed, after the enzyme solutions have been added.
- ▼ Using a dropping pipette or syringe, place a few drops of the 5% enzyme solution in the well in the first plate, be careful not to get any enzyme solution on the surface of the agar, and do not fill the hole too full.
- ▼ Using a clean pipette or syringe each time repeat step 3: with the other known solutions of enzyme. Fill the well of the control plate with boiled 5% amylase solution, and fill the wells of the other plates with the appropriate solutions.
- ▼ Leave the plates undisturbed for at least an hour, do not move them, as you may spill the solutions out of the wells.
- ▼ For all the dishes except the one with the methylene blue, flood the surface of the plates with iodine solution and leave for several seconds until the blue-black colour develops. Carefully pour off any excess iodine solution and gently rinse the agar with water.
- ▼ Examine the plates and measure the diameter of the clear zones around the wells. Recording the diameters of the clear zones produced by the known solutions should allow you to determine the concentration of the unknown solution(s) provided.
- ▼ Observe the methylene blue plate.
- ▼ Describe and explain all the results.
- ▼ Draw a graph of clear zone diameter against concentration of amylase solution, from which unknown concentrations of enzyme could be derived from the diameter of the clear zone they produce.
- ▼ Comment on this method of assaying enzyme solutions of unknown concentration.

continued

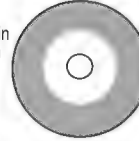
Methylene
Blue



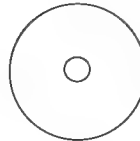
5%
Amylase
solution



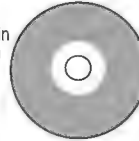
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



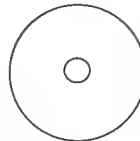
2.5%
Amylase
solution



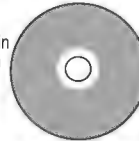
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



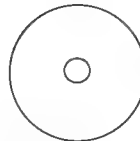
1%
Amylase
solution



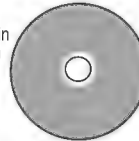
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



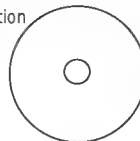
0.5%
Amylase
solution



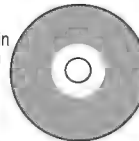
Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



Unknown
Concentration
Amylase
solution

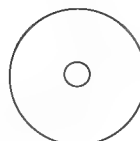


Plates flooded with Iodine in
potassium iodide solution
after 60 minutes

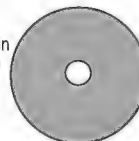


(in this case the
unknown was
an additional 2.5%
amylase solution)

Boiled
Control



Plates flooded with Iodine in
potassium iodide solution
after 60 minutes



Teaching Notes

The agar plates do not need to be sterile, nor do the students need to worry about aseptic technique, as the practical only takes an hour or so. However, you will probably use plastic petri dishes from a sterile pack, and sealed bottles of sterile agar to prepare the plates. These could be prepared beforehand and kept in a fridge for a few days.

The clear zones usually show up well in this practical, provided the students have not spilt any amylase solution onto the surface of the agar.

The methylene blue is included to demonstrate the extent of diffusion of an inert substance through the agar. It helps to counter the argument that the lack of a clear ring around the boiled enzyme is due to the fact that boiling the enzyme somehow prevents it diffusing, which is not that an uncommon assertion by students.

Requirements per student

- 7 petri dishes containing starch-agar
- Cork borer, diameter about 5 mm (sterilised by holding horizontally in the flame for 2-3 s and allowed to cool)
- Mounted needle
- Marker pen
- 7 dropping pipettes

Requirements per class

- Solutions of amylase: 5%, 2.5%, 1%, 0.1%: about 50 cm³ each
- “Unknown” solution(s) of amylase, within the range of the known solutions
- Methylene blue
- About 50 cm³ distilled water
- Iodine solution (1 dm³)

Safety Considerations

There are no safety considerations for this practical.

Genes and Genetic Engineering

2.2 The Cell cycle

Mitosis

Candidates should be able to name and explain the stages of mitosis and recognise each stage from diagrams and photographs

Preparation of root tip squash to show stages of mitosis

Introduction

An onion root tip is to be used to show the stages of mitosis in the dividing cells. The tip will need to be softened so the cells can be squashed to spread the chromosomes in one plane, and the chromosomes stained to see the stages of mitosis.

Method

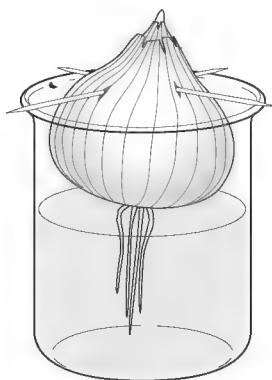
- ▼ Remove a root from a growing onion bulb. Carefully cut about 0.5cm from the tip of the root, making sure it is the tip end, not from the end near the onion. (It should be slightly pointed!)
- ▼ Place the root tip in a watch glass. Add 9 drops of acetic orcein stain, and 1 drop of 1 molar hydrochloric acid. Heat the watch glass gently, using a hotplate or spirit burner, or by passing it carefully but quickly through the flame of a bunsen burner: DO NOT LET IT BOIL! Keep the watch glass hot but not boiling for 5 minutes: if you have to hold it, use forceps.
- ▼ Place the root tip on a clean slide, and add a drop of 45% ethanoic acid. Cut away all but the terminal 1mm. Cover with a clean coverslip.
- ▼ Squash the root tip gently using a pencil: hold the pencil vertically just above the coverslip, then let it fall through the fingers onto the coverslip. Repeat this 2-3 times. This should spread the cells out under the coverslip so they can be examined under the microscope.
- ▼ Examine the slide under the microscope, using medium power (×10) objective lens and then high power (×40) objective lens. Identify the various stages of mitosis, and make annotated drawings of each stage.
- ▼ Record the final magnification of the drawing:
$$\text{eyepiece magnification} \times \text{objective magnification} \times \text{size difference}$$

Teaching Notes

Growing the onion roots needs to be started 6-7 days before the practical. It is best to use onion sets from a garden centre, or locally grown onions, as some onions bought from a supermarket have been treated to prevent sprouting.

Push two cocktail sticks through the onion at right angles: these will support the onion in the beaker, as shown in the diagram. Fill the beaker with water until it just covers the root area of the onion, and leave it in a cool place until the roots have sprouted. They do not have to be kept in the dark. Ideally, the roots need to be about 2 - 5 cm long.

If the onions are ready before the lesson is due, or if you wish to harvest onion roots and store them until needed, they can be treated with Carnoy's solution (6 parts absolute ethanol: 3 parts chloroform: 1 part glacial acetic acid) for 24 hours, then stored in 70% ethanol until needed.



The watch glasses need to be heated gently for five minutes, but they should not be allowed to boil or dry out. If they begin to dry out, an extra drop of stain can be added.

If the students have trouble finding mitotic cells in their specimen, check that they have actually used the root tip: if you can see developing xylem or phloem, they have retained the wrong portion.

It can be useful to have a few prepared slides of root tip squashes or sections, in case the students have difficulty with the slide preparation. Appropriate photographs and / or diagrams will also be useful. In addition, it will be useful if the students can see video footage of mitosis. It is important to make it clear that mitosis is a continuous process, which is subdivided for convenience.

It might be possible for the students to estimate the relative length of each stage of mitosis, by counting how many cells they can see which are in each stage.

continued

Requirements per student

Watch glass
Forceps, scalpel/knife
Slide and coverslip
Bunsen burner/spirit burner
Microscope

Requirements for class

Acetic orcein stain in dropping bottles
1 molar hydrochloric acid in dropping bottles
45% ethanoic acid, in dropping bottles
Adjustable hotplate, if available
Beaker of detergent for the used slides
Growing onions: see previously
Book illustrations, photos etc.

Safety considerations

The Acetic orcein stain, ethanoic acid and the 1 molar hydrochloric acid all need to be treated with care, as they are corrosive. You may want to provide disposable gloves for the students.

Use of dropping bottles for the staining compounds makes the practical safer.

Care is needed with heating the watch glass: a spirit burner or adjustable hotplate is better, but a bunsen burner can be used if necessary. The students should just pass the watch glass through the roaring flame, turned down low.

2.1 Transport systems

Mammalian heart

The structure and function of the heart, including the atria and ventricles, atrioventricular and semilunar valves.

Dissection of the Mammalian Heart

Method

You will be provided with a fresh or preserved sheep's heart. Examine the outer surface of the heart and identify the main blood vessels, and the position of the four chambers of the heart.

- ▼ Make sure you are looking at the ventral surface of the heart. Trace the position of the coronary artery and vein on the surface of the ventricles, starting at the opening of the artery from the aorta, and the entry of the vein into the right atrium respectively.
- ▼ Carefully cut open the two ventricles.
- ▼ Examine the interior of the right ventricle, and identify the tricuspid valve between the right atrium and the right ventricle. How many flaps does it have? Also examine the tendinous cords which are attached to the edges of the tricuspid valve. What tissues do you think they are made of, and what is their function? Look at the column-shaped papillary muscles, to which the cords are also attached. Note any congealed blood that may be present.
- ▼ Look for the semi-lunar valve leading to the pulmonary artery. Why do you think it is called a semi-lunar valve?
- ▼ Now examine the left ventricle. How does the bicuspid valve differ from the tricuspid valve? What difference can you see between the walls of the right and left ventricle, and why is this important?
- ▼ Attach a piece of rubber tubing to a water tap, and use it to run water into the blood vessels at the top of the heart (if they are present). By checking which ventricle the water enters, identify which are the venae cavae, and which are the pulmonary veins.
- ▼ Now turn the heart over and run water into the top of the aorta: what happens to the semi-lunar pocket valve in the aorta? Repeat the process with the pulmonary artery. How do these two pocket valves work?
- ▼ Run water into the right ventricle through the slit you have opened up. What happens to the tricuspid valve?
- ▼ Carefully cut into the right atrium and examine the tricuspid valve again. Cut into the left atrium and examine the openings of the pulmonary veins. Do they have valves in them?
- ▼ Now cut down the aorta from the open end and spread it out slightly. Examine the semi-lunar pocket valves from the other side. Also look for the opening of the coronary artery just above the semi-lunar valve.
- ▼ Compare the thickness and elasticity of the aorta and pulmonary artery with that of the pulmonary veins and venae cavae (if enough of these vessels remain). How is any difference in structure related to the functions of the two main types of blood vessel?

Teaching Notes

Preserved sheep's hearts can be obtained from biological suppliers. If you prefer to work with fresh materials, they can be obtained from a local abattoir or butcher, although the atria and major blood vessels and even the ventricles may be damaged. Pig's hearts may be used as an alternative.

Requirements per student

- Dissecting instruments and dissecting tray
- Disposable gloves
- Rubber tubing
- Access to a tap
- Sheep's heart

Safety Considerations

The main concern is care with the dissecting instruments, There is no risk of infection, provided that care is taken that the students do not cut themselves. Wearing disposable gloves is essential.

Physiology and Transport

2.1 Transport systems

Blood vessels

The structure of arteries, arterioles, veins and capillaries related to their functions

The observation of sections of blood vessels under the light microscope

Introduction

The observation of these vessels under the light microscope is typically of cross or transverse sections (T.S.), although capillaries are more likely to be cut in other ways in a cross section of the tissue in which they are found. The appearance of the detail will vary with the staining technique.

The artery and vein are usually straightforward to identify, although sometimes the vein is mistaken for a large space in the tissue. It may, however, be difficult to distinguish between the various tissues in the vessel wall. Capillaries are difficult to identify with any certainty in cross section at this level. Perhaps the best way is to observe a T.S. mammalian lung, identify an alveolus, then look for the capillaries that surround it. The nuclei of the endothelial cells and the sectioned red blood cells may also help in identification.

Method

- ▼ Examine a prepared slide showing a section through a mammalian artery and vein under the microscope, using the magnification you find most suitable.
- ▼ Draw a plan of each vessel, showing only the layers of tissues, and label the drawing.
- ▼ Record the final magnification of the drawing:
Eyepiece magnification $\times$ objective magnification $\times$ size difference, and write a suitable heading.
- ▼ Now examine a prepared slide of a section of mammalian lung tissue, and try to identify the alveoli and capillaries next to them, using high power ($\times 40$) objective lens. The capillaries are lined with an endothelium of very thin squamous cells, but you will be probably only be able to see their stained nuclei. The capillaries may contain red blood cells, cut in section.
- ▼ If possible make a drawing of the capillary; enlarge it as much as you can.
- ▼ Label the drawing and record its final magnification. Do not forget the heading.
- ▼ Draw a suitable table and complete it to compare the structural similarities and differences between the three types of blood vessel.

Teaching Notes

Details of the relative sizes are important to show the variation in sizes of these three types of vessel.

Measurement of the artery, vein and capillary would give a good opportunity to use the eyepiece graticule.

Requirements per student

Microscope

Prepared slide of a T.S. mammalian artery and vein

Prepared slide of a T.S. mammalian lung tissue

Drawing materials

Reference books/Photographs and/or diagrams of appropriate tissues

Safety considerations

There are no safety considerations for this practical other than the safe use of the microscope.

Physiology and Transport

2.2 The control of breathing and heartbeat

Control of heartbeat

Response of the heart to increased muscular activity

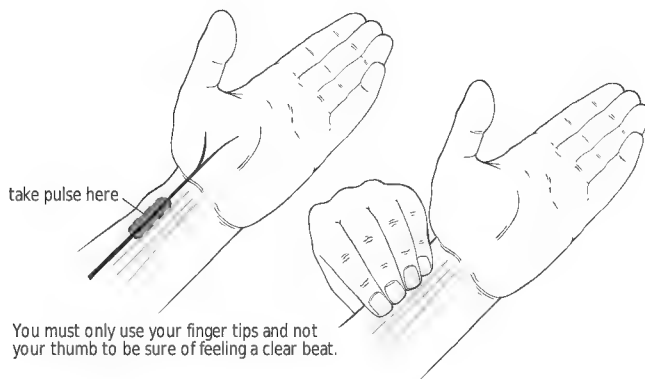
Measuring the resting pulse rate

Introduction

The pulse rate is the product of the heart rate, and can be taken to be the same thing. As the ventricles contract blood is forced out into the arteries, the walls of which expand to accommodate the surge of blood from the heart. When the ventricles relax the elastic walls of the arteries recoil until the ventricles contract again in the next cardiac cycle.

Method

The pulse can be felt in the radial artery at the wrist as shown.



-
- ▼ Allow the subject to settle for two minutes (whilst standing).
 - ▼ During this time locate the pulse in the left wrist as illustrated (do not use your thumb).
 - ▼ After 2 minutes count the pulse beats for 30 s, and record the result in a suitable table.
 - ▼ It is possible to count for a shorter period e.g. 6 s, but when measuring the resting pulse rate the longer the counting time, the more accurate is the conversion to beats per minute (*bpm*)
 - ▼ At the end of counting, leave the subject at rest for a further 2 minutes (whilst standing).
 - ▼ Repeat the 30 s pulse count procedure, and record the result.
 - ▼ Allow to rest for 2 minutes (whilst standing).
 - ▼ Repeat the 30 s pulse count procedure, and record the result.
 - ▼ Convert each result to beats per minute and work out the average resting pulse rate per minute.

An investigation into the effect of exercise on the pulse rate (heart rate)

Method

In a group of three: A will exercise, B will check the pulse, C will time and record.

- ▼ Before exercise is started, make sure that all members of the group have submitted a prior consent form.
- ▼ Subject A should rest for 2-3 minutes, standing, then subject B checks their resting pulse in beats per minute, and C times and records. It is best to check the resting pulse rate three times for each individual, and calculate the average.
- ▼ Subject A should then exercise at a steady rate for three minutes. This exercise can be using an exercise bike, or jogging on the spot, or stepping up and down using a box or the stairs. It is important that the exercise should be at a steady rate, which is one that the entire group can keep up with: it is probably a good idea if the least fit member of the group goes first.
- ▼ Immediately after the exercise, B should count subject A's radial pulse rate, while C keeps track of the time and records the results. Count the pulse rate for 30 s, then multiply the results by two. Subject A should not try to count their own pulse, as this can influence their results.
- ▼ Continue to count pulse rate for 30 s of each minute for four minutes, while subject A rests (standing). Do not stop the clock between measurements: by counting for 30 s only you should have time to record the results for each minute before you start the next.
- ▼ Change roles within the group, and repeat the process (steps 2-5) for each student in the group, so you have all exercised, and recorded results etc.
- ▼ If you have time, repeat the whole process again for all members of the group, to check the reliability of the results.
- ▼ Present the results in an appropriate way, and in the report consider the different results from members of the group, and see if you can relate them to different levels of fitness.
- ▼ Evaluate the evidence and method.

Teaching Notes

Generally the resting pulse rate is taken when the subject is flat on their back. However, where the intention is to investigate the effect of exercise on the pulse rate, it may be better to measure the 'resting' pulse rate standing, otherwise there is an increase in the pulse rate associated with getting up to participate in the exercise.

In recent years, electronic heart rate monitors (HRM's) have become more readily available. Clear advantages offered by HRM's over manual pulse-taking are that heart rate can be continuously measured over extended periods of time, and also during a wide range of physical activities which do not lend themselves to periodic bouts of wrist holding. The range of the transmitter is about 1metre; this is not a great distance but it does not usually create any practical problems. The receiver, depending on the model and the cost, may give a simple digital display of current heart rate, or it may be capable of storing heart rate data in a number of files which can be retrieved either manually or down-loaded to a PC.

Strictly speaking it is not possible to obtain the true resting pulse rate in a classroom or a laboratory, so students could be given instruction in taking their pulse rates as they wake in the morning. Taking this truly resting pulse on, say, four consecutive mornings and then taking a mean is a satisfactory method. Resting heart rate can be taken as an indicator of the subject's status, for example some advise that it is best not to train if the resting heart rate is 10% above the normal rate for that person.

To investigate the effects of exercise on the body it is necessary to establish a standard rate of exercise, during and immediately after which it is possible to record various measurements of the effect of exercise.

The validity and reliability, and relative safety of sub-maximal and maximal tests should be understood.

The ability to extrapolate from sub-maximal results to maximal results, and the correlation between various measures of body response to exercise, will all ensure a scientific approach to the investigation.

A cycle ergometer, heart rate monitor, and stethograph allow accurate and reliable recording, but are beyond the reach of many. For those without access to these, the **Step Test**, along with simple measuring of e.g. heart rates, pulse rates breathing rates, temperature, and even composition of expired air can still produce reasonably valid and reliable results.

Sub-maximal tests like the Step Test pose less risks for the subjects and the responsible supervisor than do maximal tests like the shuttle run, or those that can be performed on the cycle ergometer.

The step test involves stepping up and down a measured height at a controlled rhythm, requires the minimum of apparatus and space, and can be administered to large numbers at the same time. The stepping height can be adjusted for individuals, for example from between 10 cm and 50 cm, depending upon the height and weight of the subject. The stepping rhythm is established with the aid of a pre-recorded signal, of so many steps per minute (22 for females and 24 for males). There is always the risk of stumbling, so care must be taken.

continued

The pulse rate can be taken before, during and after a standard stepping period (usually 3 to 5 minutes). Once the heart rate has returned to its pre-exercise resting rate, the work period can be repeated if necessary with increased effort, for example by increasing the mass with added weights, and/or the rate of stepping.

Step tests are theoretically as valid (they should measure what they claim to measure) and reliable (they should do so consistently) as cycle ergometer tests. However, they rely on doing work by raising the body weight against gravity at a set rate, and their validity and reliability depends on correct stepping technique which is difficult to maintain. Any change in the height due to poor leg extension, or in the rate of stepping during a test, will change the work rate, usually reducing it, and this will invalidate the results. Stepping efficiency is also affected by the length and proportions of the legs.

SYKES, K. (1987) *Fitech Step Test*. Fitech Ltd. Alan House, 17-19 Boughton Road, Chester, CH3 5AE.

Also see: HEYWARD, V. H. (1991) *Advanced Fitness Assessment and Exercise Prescription*. Human Kinetics Books, Champaign, Illinois.

This can be a straightforward practical exercise, which can give some interesting results, with a minimum of apparatus needed; especially if the age, sex or level of fitness varies within each group. It is usually best if they work in groups of three, this provides 1 subject, 1 to count pulse rate, and 1 to check timing and record results.

You need to make sure students are checking the pulse correctly, and that they have made the practical as valid and fair as possible, with equal levels of exercise, etc.

Equipment

Step boxes, bench, safe stairs, or exercise bike
Stopclocks

Safety Considerations

The subject should be in good health and no pressure must be exerted on the subject to take part in the test activity, neither should any element of competition be allowed to enter the proceedings.

It is advisable to ask the parents/guardians of the students to complete a prior consent form (or the students themselves if they are over 18) before asking them to be subjects for experiments: a sample copy is provided.

Sample Prior Informed Consent Form

Title of Investigation/Experiment:.....

Objectives:.....

Description of Procedure:.....

Questions: All questions relating to the investigation or experiment will be answered in full.

Safety: All the procedures to be used in this investigation/experiment are widely and commonly used in Schools and Colleges, and although all activity involves some risk of an accident, the risk of harm in this investigation/experiment is no greater than in normal everyday life, or than in a supervised practical lesson.

Right of Withdrawal: You are absolutely free to withdraw from the investigation/experiment at any time. If during the investigation/experiment you feel in any way uncomfortable and wish to stop, you should do so.

Reassurance of Confidentiality: All information obtained as a result of the investigation/experiment will be treated confidentially.

Availability of Results: The data may be recorded manually, and/or stored on a computer, but not in association with your name. You are entitled to a copy of your own results on request.

Use of Results: The data may be used in discussions and/or as part of assignments or projects, but not in association with your name. They will only be used to illustrate general physiological principles, and will not be used for diagnostic or any other purposes

Statement of Consent: I fully understand the nature of the tests, and agree to participate in this investigation/experiment. To the best of my knowledge I suffer from no illness or condition that will:

i prevent me from successfully completing the tests,

ii be made worse in anyway by my taking part in the tests.

Name of Subject:.....

Signed:.....

Date:.....

Name of Parent or Legally Authorised Representative:

.....

Signed:.....**Date:**.....

Physiology and Transport

3.4 The transport of substances in Plants

Uptake and the transpiration stream

Transpiration and the effects of light, temperature, humidity and air movement

An investigation of factors affecting the rate of transpiration using the potometer

Introduction

Transpiration can be measured using a potometer. The principle of which, is that as the water evaporates from the leaves of a shoot, water is pulled up the shoot by the cohesive pull of the transpiration stream, and an air bubble is drawn along a capillary tube at the same rate - thus giving a measure of the rate of transpiration.

The time taken for the air bubble to travel a certain distance, or the distance travelled in a certain time, can be recorded and the rate of transpiration calculated.

If placed on a sensitive electronic balance, the loss of mass represents the loss of water by transpiration from the leaves.

If a set of consistent results is obtained, the figures of the uptake and of the loss of water, can be compared.

Method

- ▼ The potometer is filled by immersing it totally in water, preferably freshly boiled and cooled water which has no air bubbles.
- ▼ The shoot to be used should be cut from the plant under water, to prevent any air bubbles entering the xylem elements, and kept under water until it is inserted into the apparatus
- ▼ All joints in the apparatus should be well vaselined to prevent any leaks, which would invalidate the results by reducing the apparent rate of transpiration.
- ▼ After setting up, but before allowing air to be drawn into the capillary tube, the apparatus should be allowed to come into equilibrium with the surroundings, with the end of the capillary tube being kept under water until such time as an air bubble is introduced.
- ▼ An air bubble is introduced by lifting the capillary tube out of the water to allow air to be drawn up, and then returning the tube to the water.
- ▼ The time it takes for the bubble to travel a set distance along the tube is then recorded.
- ▼ After the bubble has travelled a certain distance, it is returned to the beaker end of the tube by running water in from the reservoir.
- ▼ The procedure is repeated until some consistent results are obtained.

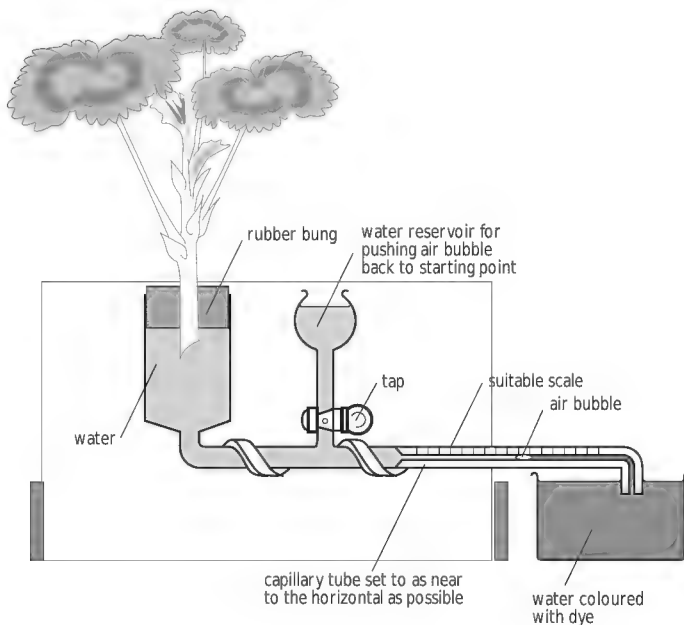
continued

▼ If the calibration on the capillary tube is in millimetres, and the diameter and hence the radius of the tube is known, then the volume of water taken up can be calculated using the formula:

$$\pi r^2 \times \text{distance travelled by bubble} = \text{volume of water uptake}$$

Sometimes, however, the calibrations are already in units of volume, for example mm^3 or cm^3 (ml).

Express the results as either cm^3 per minute, cm^3 per hour, or cm^3 per hour per cm^2 of leaf area.



Teaching Notes

The experiment is often run without a bubble, just with the end meniscus as the point of measurement. Strictly speaking this is less valid than using a bubble, as it is possible for water to evaporate from the open meniscus, thus increasing the apparent rate of water uptake - although in reality the effect is immeasurable.

The potometer actually measures the rate of water uptake, which under these experimental conditions is not the same as the transpiration rate. In this experiment the cross sectional area of the cut shoot is not as large as the equivalent area of root hairs that would normally supply the leaves, therefore the rate of uptake is less than the rate of transpiration.

Lengthy attempts to set up a whole plant complete with its root system in a potometer, in order to increase the rate of movement of the meniscus, and overcome the problem of the cut stem not providing enough surface area for water uptake, all failed for unknown reasons.

If set up successfully the apparatus can be used to compare rates of transpiration in different environmental conditions. e.g. still air and moving air (possibly using a hair dryer or fan).

The apparatus could also be set up with different leaf surfaces greased.

Given ideal results, it would be found that the loss of water as determined by placing the apparatus on an electronic balance for the duration of the experiment, is greater than the amount of water uptake as determined by the movement of the bubble.

Requirements per group

Potometer set up and ready to go
or
Potometer
Vaseline
100 cm³ beaker, water and dye
Large sink
Suitable leafy shoot cut in the correct way, and to fit the apparatus
Hair dryer or fan if required

Safety Considerations

There are no special safety considerations for this experiment, only the normal precautions with the cutting of the shoot and using the hairdryer or fan.

Physiology and Transport

3.4 The transport of substances in Plants

Uptake and the transpiration stream

Transpiration and the effects of light, temperature, humidity and air movement

Water loss from leaves

Introduction

The loss of mass from leaves will be measured. It is assumed that any loss of mass that occurs is caused by transpiration, i.e. evaporation of water through the stomata of the leaf. A count of the number of stomata on the two surfaces of the leaf will be taken and related to the rate of transpiration.

Method

- ▼ Collect 4 large leaves, with the leaf stalk attached, from the plant material provided. Find some way of identifying which leaf is which, e.g. by tying coloured thread around the stalk of each leaf, leaving long ends which you can use later. Do not write on the leaf surface with pens or markers.
- ▼ Dip the ends of all the leaf stalks in Vaseline to seal them. This prevents water loss from the cut ends.
- ▼ Each group should tie a length of string between two stands or two taps, so that the leaves can be suspended in the air, to allow free movement of air around them.
- ▼ Treat the four leaves as indicated below:
 - A: Leave untreated as a control;
 - B Smear the whole lower surface of the leaf with Vaseline: do not get any on the upper surface;
 - C Smear the upper surface of the leaf, in a similar way;
 - D Smear both surfaces.
- ▼ Tie the cotton ends of each leaf to a paperclip, and bend the wire end of the paperclip out slightly so it can be used to hook the leaf over the string support. Weigh each leaf, once it has been treated and the clip attached, and note the mass of the treated leaf (cotton, clip and all!).
- ▼ Hook each leaf over the string using the paperclip, so they are separated and air can flow freely around them. Try to ensure there is no draught around the leaves. Start the stopclock.
- ▼ At 20-minute intervals, re-weigh each leaf in turn and note the new mass. When you are moving the leaves, be careful not to touch them if you can avoid it. Handle the paperclip only. Continue to weigh the leaves every 20 minutes for 1 - 2 hours.

continued

-
- ▼ Record the results in a suitable table and calculate the percentage loss of mass for each leaf (remember to remove the weight of the paper clip from the calculations). Consider how the different treatments have affected the rate of loss of mass.
 - ▼ Repeat the experiment, using fresh leaves, and putting the leaves in a draught of warm or cool air, e.g. from a hairdryer or fan.
 - ▼ While the experiment is continuing, collect two fresh leaves (the same type as before), and paint a small amount of clear nail varnish on the upper surface of one leaf, and the lower surface of the other (about 1cm square in each case). Do not forget to continue the measurements of mass loss from the other leaves!
 - ▼ Allow the nail varnish to dry thoroughly, then carefully peel the nail varnish layer off the lower surface, using forceps. Place the layer of nail varnish on a microscope slide in a drop of methylene blue, and make sure it is lying flat. Place a coverslip over the nail varnish layer. The nail varnish makes an imprint of the cell layer on the surface of the leaf, so you can see the surface layer without having to peel the epidermis itself off the leaf.
 - ▼ Examine the lower surface imprint using the medium power (x 10) objective lens of the microscope. Identify the stomata and guard cells on the leaf surface. Count how many stomata you can see in the field of view of the microscope. Then move the microscope to another area of the layer and count how many stomata are visible.
 - ▼ Repeat the count five times, and calculate a mean number of stomata per field of view.
 - ▼ Repeat the process with the leaf upper surface nail varnish layer, and calculate the mean number of stomata per field of view on the upper surface. How do these figures relate to the results of the leaf mass experiment?

Teaching Notes

The standard way of examining transpiration is to use the potometer. However, this is difficult to set up and get to work consistently. This alternative works well and is much easier to carry out, although the criticism could be levelled against it that water loss from a detached leaf will be very different from one with a water supply.

Provided the plant material is freshly cut, or ideally provided as a pot plant, this experiment works well and is simple to set up.

If you have enough plant material, it would be best for each group to use 3-4 leaves for each treatment. This would give a larger and more measurable mass loss, and more valid results.

If you are short on time, some groups within the class can work without air draughts, others with cool or warm draughts, and then they could combine their results.

It is suggested that you should set up a bubble potometer experiment for the students to see as a demonstration, so they can understand questions based on its use. Combined with the understanding of factors which affect transpiration gained from the leaf greasing experiment, this should allow them to answer any questions based on the potometer.

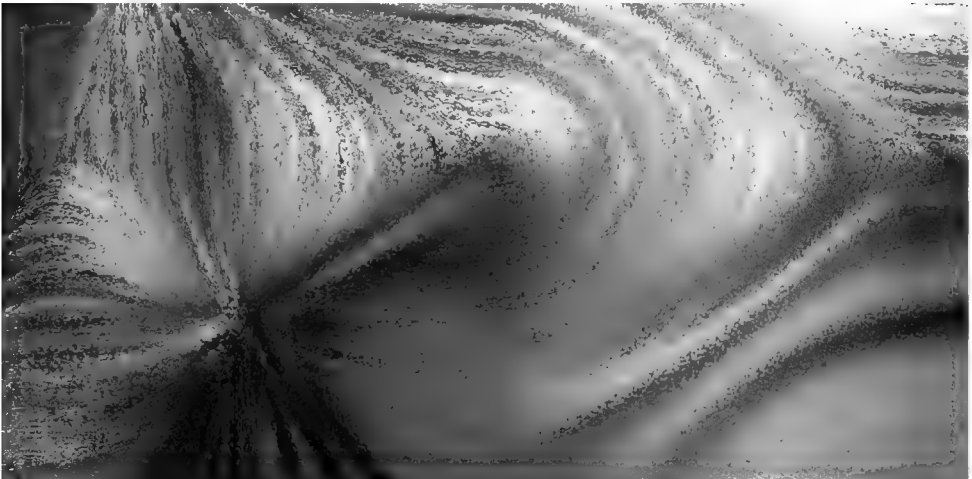
Requirements per student

- Length of string, about 1-2 m
- Lengths of cotton thread in 4 different colours
- Vaseline
- Paper clips
- Clear nail varnish
- Forceps
- Microscope
- Slide and coverslip
- Methylene blue in a dropping bottle
- Balance
- Hairdryer or fan heater
- 2 clamp stands
- Stopclock
- Supply of freshly cut plant material, from a plant with large leaves. Choose a non-hairy variety of plant.

Safety Considerations

There are no safety considerations for this experiment, apart from caution with the hairdryer or fan and the safe use of the microscope.

Exam Style Questions papers



Exam Style Questions and mark schemes



1.1 Biological Molecules

1.2 Cells

1.3 Cell Transport

1.4 Exchange of Materials with the Environment

1.5 Enzymes

1.6 Digestion

Examination Style Question Papers

Biology

Name: _____

I - Core Principles

Year Set I

Time allowed I hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

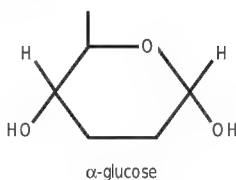
- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Section A

Answer all the questions in the spaces provided.

Question 1

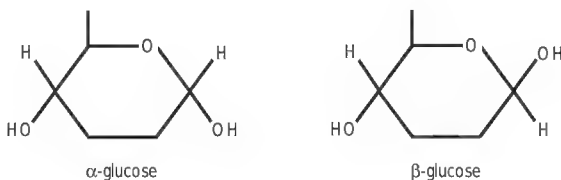
- a) The structure of glucose is shown below:



The short-hand convention used in drawing organic molecules in this way omits the carbon atoms. Indicate on the diagram above where the 6 carbon atoms of glucose are to be found.

(1)

- b) Two glucose molecules are shown below:



- i) Indicate on the diagram how the disaccharide maltose would be formed.

(1)

- ii) What type of reaction is this known as?

(1)

- iii) Name the chemical bond formed in this way.

(1)

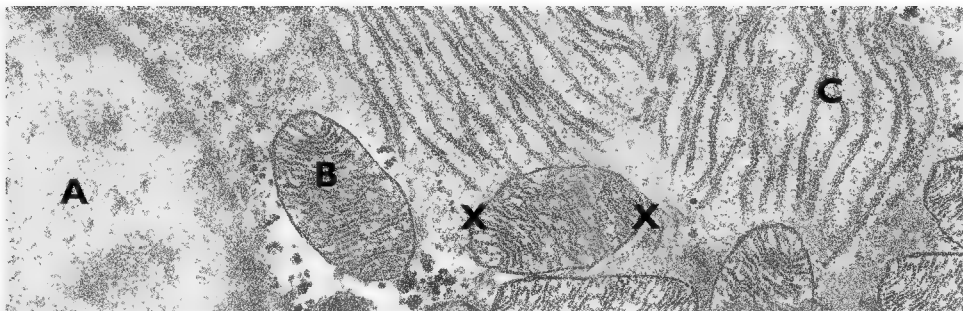
- iv) Name and describe the type of reaction that would result in the two alpha glucose molecules being regained from the maltose molecule.

(2)

Total 6

Question 2

The picture below shows part of an animal cell as seen under an electron microscope at a magnification of about $\times 25000$, with all parts shown accurately to scale.



- a) Identify structures A to C.

A _____ (1)
B _____ (1)
C _____ (1)

- b) Describe two ways in which a bacterial cell differs from the cell shown above.

i) _____ (1)
ii) _____ (1)

- c) Calculate the actual size of structure C as measured between the two points marked X. Show your workings.

_____ (3)

- d) Name a structure in the drawing that is not visible even under the best light microscope.

_____ (1)

Total 9

Question 3

The rate of diffusion across a surface is proportional to:

surface area x difference in concentration divided by thickness of exchange surface

a) Describe how each of the factors in the equation affects the rate of diffusion:

i) surface area

_____ (1)

ii) difference in concentration

_____ (1)

iii) thickness of exchange surface

_____ (1)

b) What feature of a mitochondrion increases its internal surface area?

_____ (1)

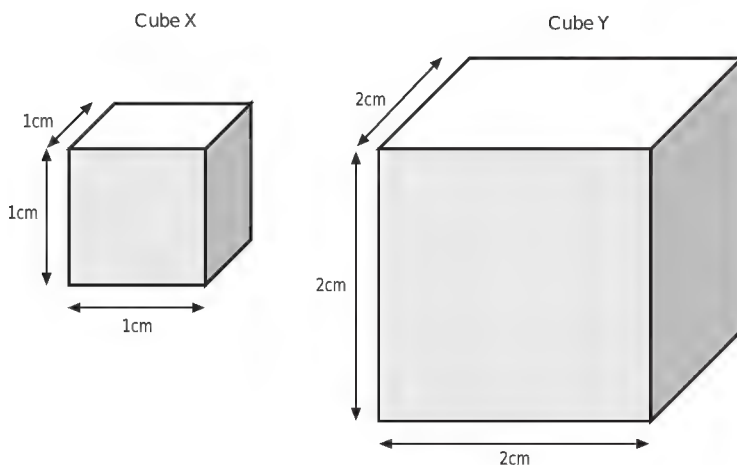
c) Explain how an increase in the aerobic activity of a cell increases the diffusion gradients of oxygen and carbon dioxide between the cytoplasm of the cell and the internal matrix of a mitochondrion.

_____ (4)

Total 8

Question 4

The diagrams below show two cubes with their dimensions.



- a) Calculate the surface area to volume ratio of the two cubes shown above.
- _____
- _____
- _____ (2)
- b) Describe how the surface area to volume ratio of a cube could be increased.
- _____ (1)
- c) i) Explain the significance of the surface area to volume ratio for the exchange of substances and of heat between organisms and their environment.
- _____
- _____
- _____
- _____ (4)
- ii) Name two surfaces in the human body which are specialised to present a large surface area for exchanges.
- _____ (1)

Total 8

Question 5

- a) i) Give a definition of the term catalyst.

_____ (1)

- ii) Name an enzyme and the reaction that it catalyses.

_____ (1)

- b) What is meant by the term 'enzyme specificity'?

_____ (1)

- c) Enzymes are denatured by extremes of pH. Explain what is meant by the term 'denatured'?

_____ (2)

Total 5

Section B

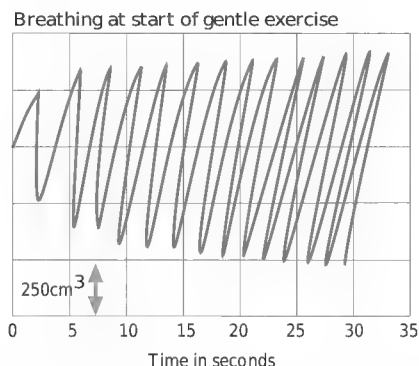
Answer all the questions in the spaces provided.

Answers should be written in continuous prose wherever appropriate.

Quality of written communication will be assessed in these answers.

Question 6

The diagram below is of a trace from a respirometer measuring the breathing of a person at rest who, then starts exercising at a rate which causes them to start breathing more heavily and quickly.



- a) Ventilation of the lungs is by tidal flow in and out of a single air tube the trachea. What are the main disadvantages of this method of ventilating a respiratory system?

(2)

- b) i) Calculate the tidal volume at rest from the trace in the diagram above.

(1)

- ii) Calculate the breathing rate at rest.

(1)

- iii) Calculate the volume of air moved through the system per minute during exercise.

(2)

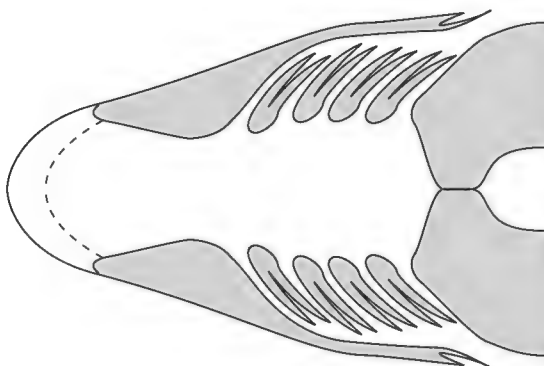
Question continued...

- c) The surfaces of the alveoli are covered in a layer of water and mucus. Does the existence of this layer of water and mucus increase or decrease the rate of gaseous exchange between the blood and the alveolar air? Explain your answer.

(1)

- d) In contrast to the tidal flow seen in lung ventilation, fish gills are ventilated by a through flow system.

- i) On the diagram below of a longitudinal horizontal section through the head of a fish in the region of the gills, draw arrows to represent through flow of water over the gills.



(1)

- ii) Also indicate on the diagram above, by means of dotted arrows, the direction of movement of water external to the fish, when the pressure in the buccal cavity decreases.

(1)

- e) Using the information above and your own knowledge, describe which one of the two systems (lungs or gills) is the most efficient for the exchange of oxygen and carbon dioxide with the environment.

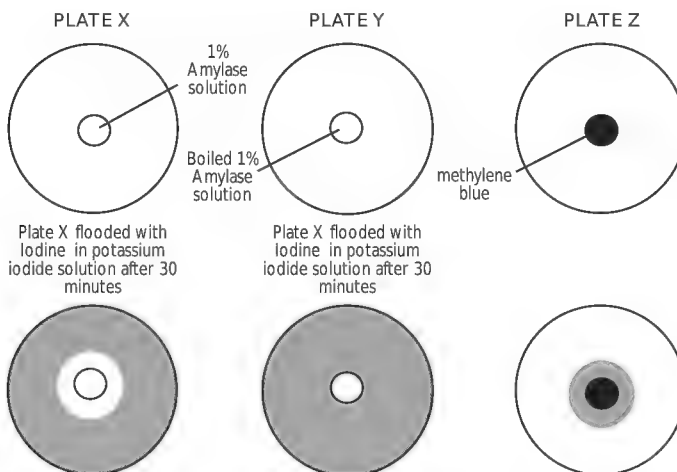
(6)

Total 15

Question 7

Drawings of starch agar plates in which circular holes or 'wells' have been made and filled with various solutions are shown below.

(Methylene blue is used in this experiment as a simple dye colour)



- a) Explain why plate X and Y were flooded with iodine in potassium iodide solution after 30 minutes.

(2)

- b) Explain why, in the flooded plate X, there is a clear area around the well containing the enzyme after 30 minutes.

(3)

- c) Explain why, in the flooded plate Y, there is no clear area around the well containing the enzyme after thirty minutes.

(3)

Question continued...

-
- d) i)** Measure the diameters of the circles surrounding the original wells in plates X and Z in the bottom row, and record your answers below.

(1)

- ii)** Using the results from section (d) i) describe the purpose of the plate with the well containing methylene blue

(5)

Total 15

Assessment Grid

Biology

I - Core Principles
Year Set I

	Question Number							
Specification Section	1	2	3	4	5	6	7	Total
10.1 Biological molecules	6							6
10.2 Cells		9						9
10.3 Cell transport			8					8
10.4 Exchange of materials				8		15		23
10.5 Enzymes					5			8
10.6 Digestion							12	12
								Total 66

	Question Number							
Assessment Objective	1	2	3	4	5	6	7	Total
AO1								
Knowledge with Understanding	5	6	4	5	5	7	9	41
AO2								
Application/Analysis/Evaluation	1	3	4	3		8	6	25
								Total 66

Mark Schemes for Question Paper

Biology

I - Core Principles

Year Set I

Instructions

; = 1 mark / = alternative response

Section A

Question I

- a) The structure of glucose is shown below:

The short-hand convention used in drawing organic molecules in this way omits the carbon atoms. Indicate on one of the diagrams above where the 6 carbon atoms of glucose are to be found.

all 6 carbons accurately marked; (1)

- b) Two glucose molecules are shown below:

- i) Indicate on the diagram how the disaccharide maltose would be formed.

removal of elements of water correctly indicated; (1)

- ii) What type of reaction is this known as?

condensation reaction; (1)

- iii) Name the chemical bond formed in this way.

glycosidic bond (1)

- iv) Name and describe the type of reaction that would result in the two alpha glucose molecules being regained from the maltose molecule.

hydrolysis reaction;

elements of water added;

across the glycosidic bond; (2)

Total 6

Question 2

The picture below shows part of an animal cell as seen under an electron microscope at a magnification of x25 000, with all parts shown accurately to scale.

- a) Identify structures A to C.
A nucleus;
B mitochondrion;
C rough or granular endoplasmic reticulum; (3)
- b) Describe two ways in which a bacterial cell differs from the cell shown above.
i) & ii) any two from
bacterium cell has no mitochondria;
no nucleus;
no endoplasmic reticulum/or other membrane bound organelle; (2)
- c) Calculate the actual size of structure C as measured between the two points marked X. Show your workings.
distance between points x-x = 25 mm;
magnification = x 25 000
therefore actual size = 25 divided by 25 000;
= 0.001 mm / 1.0 mm; (3)
- d) Name a structure in the drawing that is not visible even under the best light microscope.
ribosomes; (1)

Total 9

Question 3

The rate of diffusion across a surface is proportional to:
surface area x difference in concentration/ thickness of exchange surface

- a) Describe how each of the factors in the equation affects the rate of diffusion:
- i) surface area
the larger the surface area the faster the diffusion rate; (1)
 - ii) difference in concentration
the larger the difference in concentration the faster the diffusion rate; (1)
 - iii) thickness of exchange surface
the thicker the exchange surface the slower the diffusion rate/ vice versa; (1)
- b) What feature of a mitochondrion increases its internal surface area?
the folding(s) of the internal membrane / cristae; (1)
- c) Explain how an increase in the aerobic activity of a cell increases the diffusion gradients of oxygen and carbon dioxide between the cytoplasm of the cell and the internal matrix of the mitochondrion.
as the activity of a cell increases more ATP required;
more oxygen used;
and more carbon dioxide produced;
in aerobic respiration in the internal matrix of the mitochondrion;
difference between the levels of these two gases in the matrix of the mitochondrion and the cytoplasm of the cell increased; (4)

Total 8

Question 4

The diagrams below show two cubes with their dimensions.

- a) Calculate the surface area to volume ratio of the two cubes shown above.

Cube X: Surface Area = 6cm^2 Volume = 1 cm^3 S.A./V = 6

Cube Y: Surface Area = 24cm^2 Volume = 8 cm^3 S.A./V = 3

(2)

- b) Describe how the surface area to volume ratio of a cube could be increased.

by flattening the shape / folding the surface;

(1)

- c) i) Explain the significance of the surface area to volume ratio in the exchange of substances and of heat between organisms and their environment.

substances exchanged with the environment over the surface area of organisms;

to and from the volume of the organism;

therefore the larger the S.A./V. ratio;

the more exchange capacity per unit volume;

(4)

- ii) Name two surfaces in the human body which are specialised to present a large surface area for exchanges.

lung surface / gut surface etc;

(1)

Total 8

Question 5

- a) i) Give a definition of the term catalyst.
a substance which speeds up the rate of a reaction;
without itself being used up;
both needed for the mark; (1)
- ii) Name an enzyme and the reaction that it catalyses.
both needed for the mark; (1)
- b) What is meant by the term 'enzyme specificity'
an enzyme will only catalyse a specific reaction or type of reaction; (1)
- c) Enzymes are denatured by extremes of pH. Explain what is meant by the term 'denatured'
rendered irreversibly inactive;
as a result of permanent disruption of their 3 D structure / active site; (2)

Total 5

Section B

Answer all the questions in the spaces provided.

Answers should be written in continuous prose wherever appropriate.

Quality of written communication will be assessed in these answers.

Question 6

The diagram below is of a trace from a respirometer measuring the breathing of a person at rest, who then starts exercising at a rate which causes them to start breathing more heavily and quickly.

- a) Ventilation of the lungs is by tidal flow in and out of a single air tube the trachea. What are the main disadvantages of this method of ventilating a respiratory system?

stationary air results close to the respiratory surface;

diffusion gradients of respiratory gases decrease;

(2)

- b) i) Calculate the tidal volume at rest from the trace in the diagram above.
500cm³;

(1)

- ii) Calculate the breathing rate at rest.

24 complete breaths per minute;

(1)

- iii) Calculate the volume of air moved through the system per minute during exercise.

tidal volume = $3.5 \times 250 = 875 \text{ cm}^3$;

3 breaths per 5 seconds = 2625 cm^3

$\times 12$ for one minute = 31.5 dm^3 per minute;

(2)

Question continued...

-
- c) The surfaces of the alveoli are covered in a layer of water and mucus. Does the existence of this layer of water and mucus increase or decrease the rate of gaseous exchange between the blood and the alveolar air? Explain your answer.
- decrease as a result of increasing the diffusion distance between the air and the blood;
as a result of diffusion of gases being slower in solution than in air; (1)
- d) In contrast to the tidal flow seen in lung ventilation, fish gills are ventilated by a through flow system.
- i) On the diagram below of a longitudinal horizontal section through the head of a fish in the region of the gills below, draw arrows to represent through flow of water over the gills.
- arrows passing into mouth and out over gills; (1)
- ii) Also indicate on the diagram above, by means of dotted arrows, the direction of movement of water external to the fish, when the pressure in the buccal cavity decreases.
- dotted arrows moving into mouth;
and pushing opercular valves closed; (1)
- e) Using the information above and your own knowledge, describe how fish gills are more efficient than lungs for the exchange of oxygen and carbon dioxide with the environment.
- gills extract oxygen from water which has a much lower oxygen content than air;
no surface film of water therefore thinner diffusion surface;
through flow of external medium;
therefore no stationary medium / dead space;
double buccal and opercular pumps ensure almost continuous flow of water over gills
through flow enables counter current flow of medium and blood;
increases efficiency of gaseous exchange by diffusion;
as exchanges occur over the whole 'length' of the gill surface. (6)

Total 15

Question 7

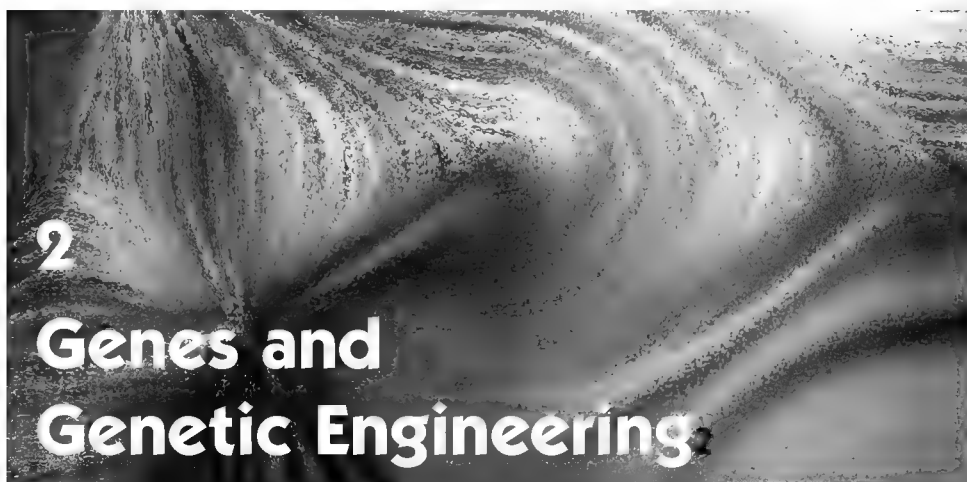
Drawings of starch agar plates in which circular holes or 'wells' have been made and filled with various solutions are shown below.

(Methylene blue is used in this experiment as a simple dye colour)

- a) Explain why plates X and Y were flooded with iodine in potassium iodide solution after 30 minutes.
to demonstrate the presence or absence of starch;
iodine in potassium iodide solution turning blue-black in presence of starch;
after 30 minutes to allow time for any reactions; (3)
- b) Explain why, in the flooded plate X, there is a clear area around the well containing the enzyme after 30 minutes.
the enzyme amylase digests starch;
diffuses out of well into surrounding starch agar;
digesting the starch as it goes;
in this area there is no starch to give a positive result with iodine solution; (3)
- c) Explain why, in the flooded plate Y, there is no clear area around the well containing the enzyme after 30 minutes.
the boiled enzyme has been denatured;
it is assumed that it has diffused out in the same way;
but has not digested starch;
therefore positive result for starch all over plate; (3)
- d) i) Measure the diameters of the circles surrounding the original wells in plates X and Z in the bottom row and record your answers below
X = 12 mm, Z = 12 mm (1)
- ii) Using the results from section d) i) describe the purpose of the plate with the well containing methylene blue
the diameters of the two circles are the same;
indicating that the rate of diffusion is the same;
for two completely unrelated chemicals (protein and dye);
therefore safe to assume that denatured enzyme diffused just the same;
rather than boiling enzyme had some other effect that stopped it diffusing; (5)

Total 15

Exam style question papers



2.1 The Genetic Code

2.2 The Cell Cycle

2.3 Sexual Reproduction

2.4 Application of Gene Technology

Examination Style Question Papers

Name: _____

Biology

2 - Genes and Genetic Engineering Year Set I

Time allowed 1 hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Question 1

- a) Give a definition of a 'gene' in terms of DNA structure.

(2)

- b) Give a definition of the term 'allele'.

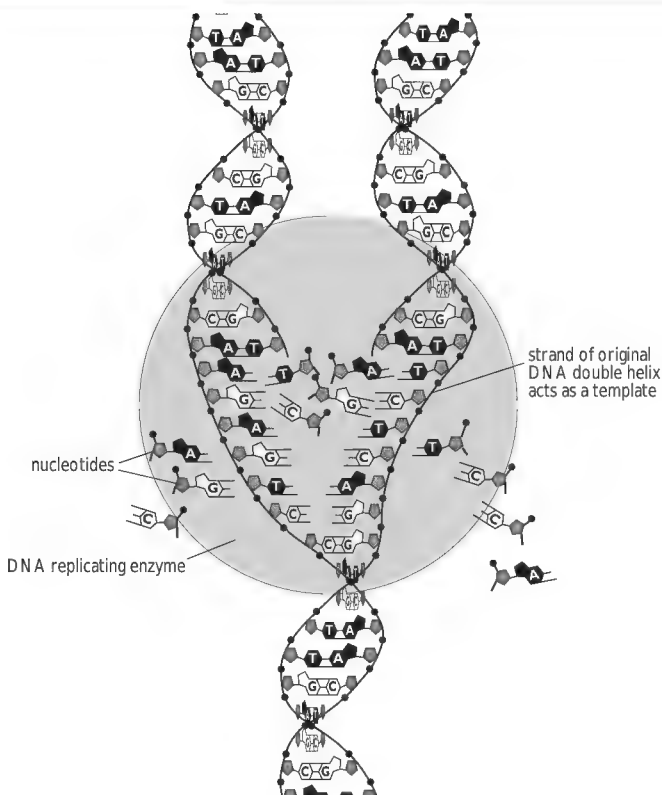
(2)

- c) Give an example of the two alleles of a named gene

(1)

Total 5

Question 2



Study the diagram of replicating DNA shown above and answer the following questions.

(a) On the diagram label the two new strands of DNA;

(1)

(b) With reference to the diagram:

i) describe what is meant by semi-conservative replication;

(2)

ii) Indicate with an arrow on the diagram the direction in which the new strands of DNA are being formed;

(1)

(c) Name an enzyme involved in catalysing the chemical reactions of DNA replication

(1)

Total 5

Question 3

- a) The genetic code consists of a sequence of bases on a strand of DNA, and can be represented by a series of letters on a line as shown below

T A C C G T T C T A C C

- i) Describe what the letters represent, and give their names.

(2)

- ii) Draw a similar line with letters to represent the complementary strand of DNA

(1)

- iii) Draw a similar line with letters to represent the complementary strand of mRNA to the original DNA strand shown at the start of the question.

(1)

- b) Explain by reference to section (a) what is meant by the terms 'sense' and 'non-sense' with regard to two strands of DNA

(4)

Total 8

Question 4

The table below shows the genetic code on a strand of mRNA for selected amino acids.

Amino acid	mRNA codons
alanine	GCA, GCC, GCG, GCU
arginine	AGA, AGG, CGA, CGC, CGG, CGU
tryptophan	UGG
lysine	AAA, AAG
methionine	AUG (also acts as the 'start' codon)

Using the information in the table give answers to the following:

- a) explain what is meant by describing the genetic code as 'degenerate';

(2)

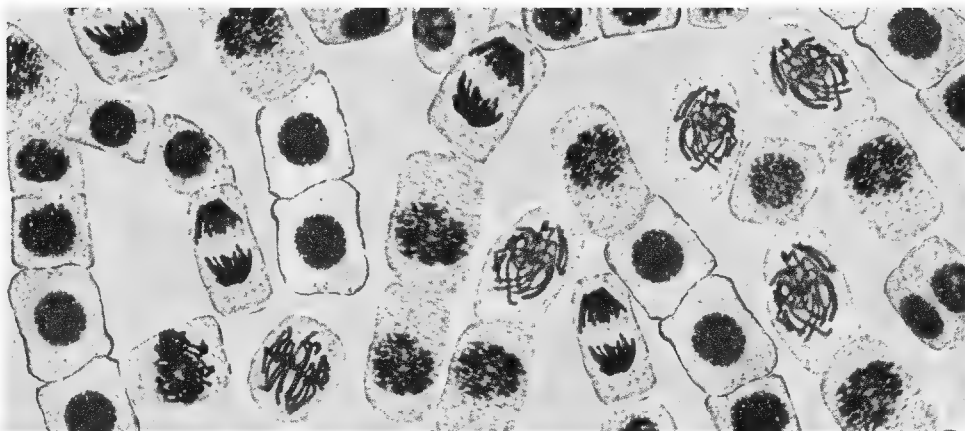
- b) Explain why every polypeptide chain begins with the amino acid methionine

(2)

Total 4

Question 5

Study the illustration below of stained cells of a squashed root tip from a plant, showing various stages of mitosis, and answer the following questions.



- (a) i) Indicate with an X a cell which is showing separation of chromatids.

(1)

- ii) Indicate with a Y a cell which is showing the chromosomes lining up on the equator of the nuclear spindle.

(1)

- (b) Explain why the specimen was squashed and not sectioned.

(3)

- (c) Explain why plant root tips are good source of cells undergoing mitosis.

(1)

Total 6

Question 6

a) Briefly describe the reactions catalysed by the following enzymes.

i) reverse transcriptase

(2)

ii) restriction endonuclease

(2)

iii) ligase

(2)

(b) In the context of gene technology explain the meaning of the term 'vector'.

(2)

Total 8

SECTION B

Question 7

Sickle cell anaemia is a blood disorder resulting from a single gene mutation which causes a change of just one amino acid in the beta polypeptide chain of the haemoglobin molecule. This is shown in the diagram below.

Section of beta chain of normal haemoglobin molecule:

GA - GA - P - T - L - H - V

Section of beta chain of 'sickle' haemoglobin molecule:

GA - V - P - T - L - H - V

Key	Amino acid	Triplet codons
V	Valine	GUA, GUC, GUG, GUU
P	Proline	CCA, CCC, CCG, CCU
T	Threonine	ACA, ACC, ACG, ACU
GA	Glutamic acid	GAA, GAG
L	Proline	CCA, CCC, CCG, CCU
H	Histidine	CAC, CAU

(a) i) Identify the 'wrong' amino acid in the mutant polypeptide chain.

(1)

ii) Identify the way in which a triplet codon for the 'normal' amino acid could mutate by a single nitrogenous base to code for the 'wrong' amino acid.

(2)

iii) Name the type of gene mutation that you described in section ii).

(1)

Question continued...

iv) Explain how the change of only one amino acid in a polypeptide chain can have such a profound effect on the haemoglobin molecule and its function.

(2)

b) i) Give a triplet codon sequence to match the order of amino acids shown for the normal haemoglobin.

(1)

ii) Explain whether the triplet codons shown earlier in this question are from DNA or RNA.

(1)

iii) Describe what the different codons for each amino acid have in common.

(1)

c) With reference to the triplet codons shown earlier in this question explain why the genetic code is described as redundant and non-overlapping.

(6)

Total 15

Question 8

Potatoes are food storage organs (tubers) produced by the parent plant at the tips of underground stems. The parent plant dies down in the autumn, and new plants are produced from the tubers the next spring. Each potato tuber has several lateral buds ('eyes') which are capable of growing into new potato plants.

- a) i)** Explain why potato plants produced in this way (vegetative propagation) are genetically identical clones.

(3)

- ii)** Many plants grow from a single tuber, and a plant grown from a single 'eye' cut from a tuber, will produce a smaller crop than a plant that has grown from a single eye on a whole tuber. Suggest an explanation for this relationship between the size of the part that is planted and the subsequent crop.

(2)

- b)** Potatoes are very susceptible to diseases, including viruses which are spread by green fly (aphids), and after two or three years of growing a crop from its own tubers there is a drop in the yield. Explain why clones of genetically identical plants are susceptible to the build up of disease.

(4)

- c) i)** To avoid the problem described in section (b) new virus-free tubers or 'seed' potatoes grown in regions of high wind and rainfall such as regions of Scotland and Ireland must be brought in. Explain why such seed potatoes will be largely free of virus infections.

(1)

- d)** Commercial strains of potatoes have four sets of chromosomes (tetraploids $4n$). Explain why strong powers of vegetative reproduction are frequently found in plants with extra sets of chromosomes

(3)

Question continued...

-
- e) Plant breeders have managed to breed in resistance to several major pests of potato plants, enabling potatoes to be grown in areas where it would otherwise be impossible. This has been achieved despite the poor fertility of commercial potato varieties, which is due to the fact that some potato seeds can be produced without fertilisation. These seeds grow into plants that can then be crossed with disease resistant wild varieties which only have two sets of chromosomes. Illustrate this sequence of events with a simple flow diagram, representing chromosome sets as n , $2n$, and $4n$.

(2)

Total 15

Assessment Grid

Biology

2 - Genes and Genetic Engineering Year Set I

Specification Section	Question Number								Total
	1	2	3	4	5	6	7	8	
11.1 Genetic code	5	5	8	4			15		37
11.2 Cell cycle					6				6
11.3 Sexual Reproduction								15	15
11.4 Gene Technology						8			8
Total 66									

Assessment Objective	Question Number								Total
	1	2	3	4	5	6	7	8	
AO1									
Knowledge with Understanding	5	1	4	2	4	6	9	9	57
AO2									
Application/Analysis/Evaluation		4	4	2	2	2	6	6	33
Total 66									

Mark Schemes for Question Paper

Biology

2 - Genes and Genetic Engineering Year Set I

Instructions

; = 1 mark / = alternative response

Section A

Question I

- a) Give a definition of a 'gene' in terms of DNA structure.
section / length of one strand of a DNA molecule;
sequence of bases A, T C, G coding for one polypeptide;
one gene one polypeptide; (2)
- b) Give a definition of the term 'allele'.
different form of a gene;
coding for a variant of the original polypeptide;
positioned in the same relative position / locus on a homologous chromosome; (2)
- c) Give an example of the two alleles of a named gene
any named example /
melanin - lack of melanin /
etc (1)

Total 5

Question 2

Study the diagram of replicating DNA and answer the following questions.

- (a) On the diagram label the two new strands of DNA; (1)
- (b) With reference to the diagram:
- i) describe what is meant by semi-conservative replication;
each daughter DNA double helix;
has conserved one strand of the original DNA double helix; (2)
 - ii) Indicate with an arrow on the diagram the direction in which the new strands of DNA are formed
Arrow pointing down (1)
- c) Name an enzyme involved in catalysing the chemical reactions of DNA replication
DNA polymerase (1)

Total 5

Question 3

- (a) The genetic code consists of a sequence of bases on a strand of DNA, and can be represented by a series of letters on a line as shown below

T A C C G T T C T A C C

- i) Describe what the letters represent, and give their names.

nitrogenous bases;

thymine, adenine, guanine, and cytosine;

(2)

- ii) Draw a similar line with letters to represent the complementary strand of DNA.

A T G G C A A G A T G G

(1)

- iii) Draw a similar line with letters to represent the complementary strand of mRNA to the original DNA strand shown at the start of the question.

A U G G C A A G A U G G

(1)

- b) Explain by reference to section (a) what is meant by the terms 'sense' and 'non-sense' with regard to two strands of DNA

the 'sense' strand carries genetic information;

as a sequence of bases coding for a sequence of amino acids;

the complementary strand of DNA carries the 'opposite' sequence of bases;

which is non-sense as it does not code for that sequence of amino acids;

(4)

Total 8

Question 4

The table below shows the genetic code on a strand of mRNA for selected amino acids.

Amino acid	mRNA codons
alanine	GCA, GCC, GCG, GCU
arginine	AGA, AGG, CGA, CGC, CGG, CGU
tryptophan	UGG
lysine	AAA, AAG
methionine	AUG (also acts as the 'start' codon)

Using the information in the table give answers to the following:

- i) explain what is meant by describing the genetic code as 'degenerate';
most amino acids are coded for by more than one triplet codon;
changes in the triplet codons (mutations) do not necessarily result in a change of the amino acid coded for; (2)

- b) Explain why every polypeptide chain initially starts with the amino acid methionine
the code for the amino acid methionine AUG;
also acts as the 'start' codon so that every polypeptide chain begins with methionine; (2)

Total 4

Question 5

Study the illustration below of stained cells of a squashed root tip from a plant, showing various stages of mitosis, and answer the following questions.

- (a) i) Indicate with an X a cell which is showing separation of chromatids. (1)
- ii) Indicate with a Y a cell which is showing the chromosomes lining up on the equator of the nuclear spindle. (1)
- (b) Explain why the specimen was squashed and not sectioned.
squashing separates out the individual chromosomes;
in one plane so all can be seen;
sectioning would only catch parts of chromosomes; (3)
- c) Explain why plant root tips are good source of cells undergoing mitosis.
growing regions, apical meristem just behind tip produce nuclei by mitosis in all directions; (1)

Total 6

Question 6

- a) Briefly describe the reactions catalysed by the following enzymes.
- i) reverse transcriptase
formation of DNA;
from RNA (2)
 - ii) restriction endonuclease
hydrolysis at points along nucleic acid;
'cutting' it into shorter lengths; (2)
 - iii) ligase
rejoins;
'cut' ends of nucleic acids; (2)
- (b) In the context of gene technology explain the meaning of the term 'vector'.
carries the engineered gene;
into the host cell;
e.g. plasmids of bacteria / viruses; (2)

Total 8

SECTION B

Quality of Written Communication

The parts to many of the answers to the questions in Section B require continuous prose. To gain one mark for Quality of Written Communication these answers should be presented in clear, scientific English. Technical terminology should have been used effectively and should usually be accurate.

Question 7

Sickle cell anaemia is a blood disorder resulting from a single gene mutation causing a change of just one of the amino acids in the beta polypeptide chain of the haemoglobin molecule, as shown in the diagram below.

- a) i) Identify the 'wrong' amino acid in the mutant polypeptide chain.
valine (for glutamic acid); (1)
- ii) Identify the way in which a triplet codon for the 'normal' amino acid could mutate by a single nitrogenous base to code for the 'wrong' amino acid.
GAA to GUA;
adenine replaced by uracil; (2)
- iii) Name the type of gene mutation that you described in section ii).
substitution; (1)
- iv) Explain how the change of only one amino acid in a polypeptide chain can have such a profound effect on the haemoglobin molecule and its function.
change in one amino acid alters primary structure;
which in turn alters bonding between side chains etc;
altering secondary and tertiary structures;
on which activity depends; (2)

Question continued...

-
- b) i) Give a triplet codon sequence to match the order of amino acids shown for the normal haemoglobin.
any correct sequence; (1)
- ii) Explain whether the triplet codons shown previously are from DNA or RNA.
RNA has Uracil instead of Thymine; (1)
- iii) Describe what the different codons for each amino acid have in common.
within a series for a single amino acid the first two letters are the same; (1)
- c) With reference to the triplet codons shown previously explain why the genetic code is described as redundant and non-overlapping.
redundant as amino acids are coded for by more than one triplet codon;
one error in a triplet codon does not necessarily lead to a change of amino acid;
e.g. GUA and GUC both code for valine;
non-overlapping in that each codon is 'fixed';
from the start codon (not shown);
if a base contributes to one codon it cannot contribute to another; (6)

Total 15

Question 8

Potatoes are food storage organs (tubers) produced by the parent plant at the tips of underground stems. The parent plant dies down in the autumn, and new plants are produced from the tubers the next spring. Each potato tuber has several lateral buds ('eyes') which are capable of growing into new potato plants.

- a) i) Explain why potato plants produced in this way (vegetative propagation) are genetically identical clones).

potato tubers arise by mitosis from parent plant;

all tubers are genetically identical;

the many 'eyes' on each potato are genetically identical;

(3)

- ii) Many plants grow from a single tuber, and a plant grown from a single 'eye' cut from a tuber, will produce a smaller crop than a plant that has grown from a single eye on a whole tuber. Suggest an explanation for this relationship between the size of the part that is planted and the subsequent crop.

potato tubers are food stores for the next generation;

the less store available at the start of growth affects subsequent growth and crop production;

(2)

- b) Potatoes are very susceptible to diseases including viruses which are spread by green fly (aphids), and after two or three years of growing a crop from its own tubers there is a drop in the yield. Explain why clones of genetically identical plants are susceptible to the build up of disease.

there is a continuity of tissue between the generations;

which are all produced by mitosis from pre-existing tissue'

this continuity enables build up of virus numbers over the generations;

no genetic variation to enable natural selection of resistance;

(4)

- c) i) To avoid the problem described in section (b) new virus-free tubers or 'Seed' potatoes grown in regions of high wind and rainfall such as regions of Scotland and Ireland must be brought in. Explain why such seed potatoes will be largely free of virus infections.

conditions of high rain and wind deter aphids flying from plant to plant;

(1)

- d) Commercial strains of potatoes have four sets of chromosomes (tetraploids $4n$). Explain why strong powers of vegetative reproduction are frequently found in plants with extra sets of chromosomes

possession of multiple sets of chromosomes interferes with meiosis;

reduced fertility;

therefore such mutations survive more frequently in organisms with strong powers of vegetative reproduction;

(3)

Question continued...

-
- e) Plant breeders have managed to breed in resistance to several major pests of potato plants, enabling potatoes to be grown in areas where it would otherwise be impossible. This has been achieved despite the poor fertility of commercial potato varieties, which is due to the fact that some potato seeds can be produced without fertilisation. These seeds grow into plants that can then be crossed with disease resistant wild varieties which only have two sets of chromosomes. Illustrate this sequence of events with a simple flow diagram, representing chromosome sets as n , $2n$, and $4n$.

$4n$ plant $\rightarrow$ $2n$ gametes $\rightarrow$ $2n$ plants $\rightarrow$ n gametes $\times$ n gametes $\rightarrow$ $2n$ plants (2)

Total 15

Exam style question papers



3.1 Transport Systems

3.2 The Control of Breathing and Heartbeat

3.3 Energy and Exercise

3.4 The Transport of Substances in Plants

Examination Style Question Papers

Biology

Name: _____

3 - Physiology & Transport

Year Set I

Time allowed 1 hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

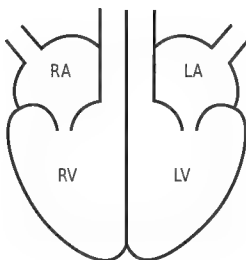
- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Question 1

The transport systems of larger organisms are closely linked with specialised exchange systems, whose main function is to maintain concentration gradients.

- a) Complete the diagram of the heart below using arrows to show the blood supply to and from the lungs. Also name the blood vessels involved

(2)



- b) The table below shows the levels of oxygen and carbon dioxide (in mm Hg) in the alveolar air and in the blood.

	Blood in pulmonary artery	Air in the alveoli	Blood in pulmonary vein
Oxygen	40.0	98	96
Carbon dioxide	46.0	40	40

Referring to the figures in the table, describe the exchange of oxygen and carbon dioxide between the blood and the air in the alveoli.

(4)

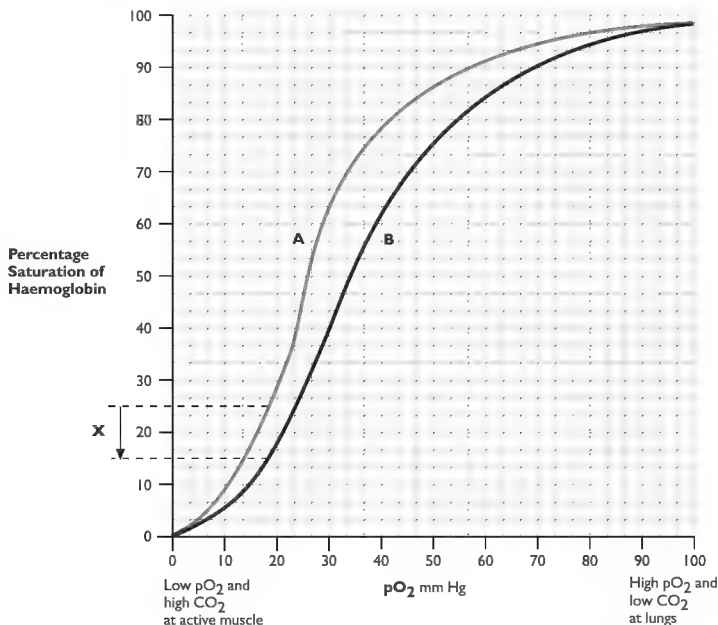
- c) Describe how the concentration gradients illustrated in the table could be increased (steepened).

(3)

Total 9

Question 2

Study the graph below showing the association of haemoglobin with oxygen at different partial pressures of oxygen, and answer the following questions.



- a) i) The two curves represent the percentage saturation of haemoglobin with oxygen at two different carbon dioxide concentrations. On the diagram label the curve which is at the higher carbon dioxide concentration.

(1)

- ii) With reference to the vertical arrow labelled X explain the significance of the difference between the two curves with respect to the delivery of oxygen to the tissues.

(3)

- b) Explain how the different conditions that exist in the case of these two curves could be increased by the activity of the body.

(3)

Total 7

Question 3

The heart responds to increased muscular activity by increasing the cardiac output.

- a) Give the simple word equation for cardiac output:

cardiac output = _____ x _____ (1)

- b) In order to respond to increasing demand the heart must receive the appropriate nervous impulses from a control centre. Name, and give the location of the structure within the heart wall that receives these nervous impulses.

_____ (2)

- c) As a result of exercise, chemicals are produced which increase the cardiac output. Name one of these chemical stimulants.

_____ (1)

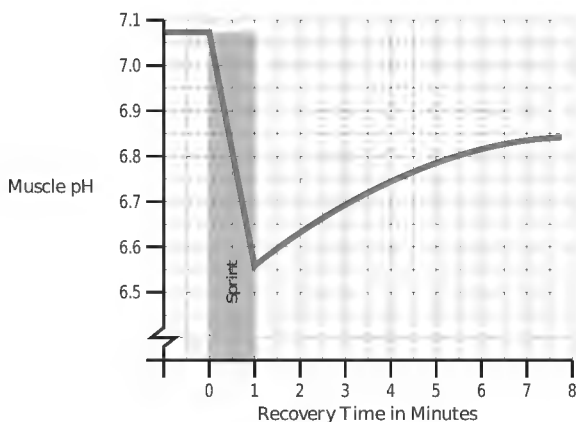
- d) State where this chemical stimulus is detected.

_____ (1)

Total 5

Question 4

The graph below shows the changes in muscle pH during a 60 second flat out running effort, and a recovery period



- a) In terms of anaerobic effort describe why the muscle pH decreases during the sprint.

(3)

- b) Describe the effect that this drop in muscle pH will have on performance during the 60 second period of effort.

(3)

- c) In terms of the removal of products of anaerobic respiration explain the recovery of the muscle pH back towards what it was before the anaerobic exercise.

(3)

Total 9

Question 5

Sorghum (the source of millet seed) grows in hot, dry conditions, and has typical xerophytic modifications, including an extensive root system, a thick cuticle, and the ability to tolerate high temperatures.

- a) Describe the role of xerophytic modifications.

(3)

- b) Describe a special feature of Sorghum cells which enable the plant to tolerate high temperatures

(2)

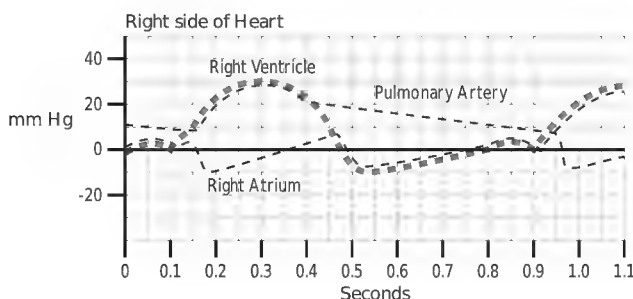
- c) Sorghum also has a reduced number of sunken stomata. Explain how these help Sorghum to survive in dry hot conditions.

(3)

Total 8

Question 6

Study the diagram below showing the pressure changes in the right side of the heart, and answer the questions that follow.



- a) i) Mark on the horizontal time axis the period during which the right ventricle is relaxing. (1)
 ii) Describe the state of the tricuspid and pocket valves during this period.

(2)

- b) Explain the negative pressure in the right atrium.

(1)

- c) i) From the graph deduce the highest (systolic) value for the blood pressure in the right ventricle.

(1)

- ii) Explain why the pressure generated by the right ventricle is necessarily much lower than that generated by the left ventricle, which is about 120 mm Hg.

(3)

Question continued...

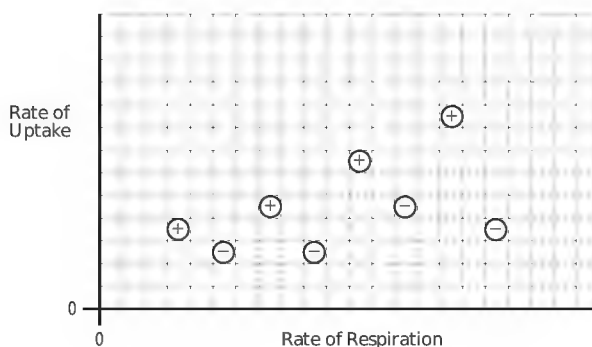
-
- d) With reference to the pressures indicated on the diagram, explain the events in the right ventricle during one complete cardiac cycle.

(6)

Total 14

Question 7

The simplified graph below shows the relationship between the rate of uptake of positively charged cations, negatively charged anions, and the rate of respiration.



- a) i) Briefly describe the relationship between the uptake of the ions and the rate of respiration.

(2)

- ii) Give a concise definition of active transport with regard to the uptake of ions by plants.

(3)

- iii) With reference to the graph, identify which type of ion is taken up more actively.

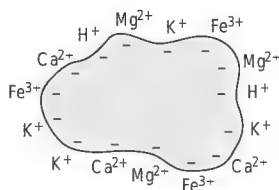
(1)

- iv) Which part of the root is most involved in the uptake of inorganic ions from the soil?

(1)

Question continued...

- b)** The diagram below represents the 'cation store' of the soil, which exists as the result of negatively charged clay particles holding positively charged cations.



- i)** The different cations are held by attractions of different strengths, and are released and made available for uptake by the roots of plants, after being displaced by hydrogen ions (H^+). Explain how the respiration of root hair cells provides a supply of hydrogen ions (H^+).

(3)

- ii)** By reference to the diagram of the cation store explain why negatively charged anions such as nitrate ions must be continuously replenished in the soil.

(4)

Total 14

Assessment Grid

Biology

3 - Physiology & Transport
Year Set I

	Question Number							
Specification Section	1	2	3	4	5	6	7	Total
12.1 Transport Systems	9	7				14		30
12.2 Breathing and heartbeat			5					5
12.3 Energy and exercise				9				9
12.4 Transport in plants					8		14	22
								Total 66

	Question Number							
Assessment Objective	1	2	3	4	5	6	7	Total
AO1								
Knowledge with Understanding	6	4	5	6	6	8	8	43
AO2								
Application/Analysis/Evaluation	3	3		3	2	6	6	23
								Total 66

Mark Schemes for Question Paper

Biology

3 - Physiology & Transport Year Set I

Instructions

; = 1 mark / = alternative response

Question I

The transport systems of larger organisms are closely linked with specialised exchange systems, whose main function is to maintain concentration gradients.

- a) Complete the diagram of the heart below using arrows to show the blood supply to and from the lungs. Also name the blood vessels involved (2)
- b) The table below shows the levels of oxygen and carbon dioxide (in mm Hg) in the alveolar air and in the blood.
- Referring to the figures in the table, describe the exchange of oxygen and carbon dioxide between the blood and the air in the alveoli.
- oxygen in blood in pulmonary artery at 40.0 lower than air in the alveoli at 98;
diffusion gradient from alveoli to lungs;
therefore oxygen diffuses into blood;
carbon dioxide in pulmonary artery at 46.0 higher than air in the alveoli at 40;
therefore carbon dioxide diffuses out of blood; (4)
- c) Describe how the concentration gradients illustrated in the table could be increased (steepened).
- by increased activity;
therefore increased oxygen consumption;
and increased carbon dioxide production;
only slightly by increased ventilation; (3)

Total 9

Question 2

Study the graph below showing the association of haemoglobin with oxygen at different partial pressures of oxygen, and answer the following questions.

- a) i) The two curves represent the percentage saturation of haemoglobin with oxygen at two different carbon dioxide concentrations. On the diagram label the curve which is at the higher carbon dioxide concentration.
- right hand curve; (1)
- ii) With reference to the vertical arrow labelled X explain the significance of the difference between the two curves with respect to the delivery of oxygen to the tissues.
- arrow X indicates difference in saturation;
at one particular oxygen level;
more oxygen released to tissues;
when right hand curve operating (high carbon dioxide); (3)
- b) Explain how the different conditions that exist in the case of these two curves could be increased by the activity of the body.
- difference depends on different levels of carbon dioxide;
levels of carbon dioxide increased by increased respiration;
eg as a result of increased exercise; (3)

Total 7

Question 3

The heart responds to increased muscular activity by increasing the cardiac output.

- a) Give the simple word equation for cardiac output:

cardiac output = heart rate x stroke volume; (1)

- b) In order to respond to increasing demand the heart must receive the appropriate nervous impulses from a control centre. Name, and give the location of the structure within the heart wall that receives these nervous impulses

sino-atrial node;

right atrium; (2)

- c) As a result of exercise, chemicals are produced which increase the cardiac output. Name one of these chemical stimulants.

carbon dioxide / lactate (lactic acid) / low pH; (1)

- d) Describe where this stimulus is detected.

directly by the cardio-vascular / vasomotor centre/medulla of brain/

carotid bodies / aortic bodies; (1)

Total 5

Question 4

The graph below shows the changes in muscle pH during a 60 second flat out running effort and a recovery period

- a) In terms of anaerobic effort describe why the muscle pH decreases during the sprint.
in absence of sufficient oxygen anaerobic pathways operate;
resulting in production of lactic acid'
which dissociates into lactate and hydrogen ions;
pH a measure of hydrogen ion concentration; (3)
- b) Describe the effect that this drop in muscle pH will have on performance during the 60 second period of effort.
inhibits muscle contraction;
by inhibiting enzyme activity;
muscle contraction slows and stops;
perception of pain reduces effort;
performance drops; (3)
- c) In terms of the removal of products of anaerobic respiration, explain the recovery of the muscle pH back towards what it was before the anaerobic exercise.
lactate removed by the blood circulation;
and the lymphatic system;
to be oxidised elsewhere eg heart muscle;
pH lowered by exhalation of carbon dioxide in the lungs;
excretion by kidneys; (3)

Total 9

Question 5

Sorghum (the source of millet seed) grows in hot, dry conditions, and has typical xerophytic modifications, including an extensive root system, a thick cuticle, and the ability to tolerate high temperatures.

- a) Describe the role of xerophytic modifications.
to take up and conserve water / efficient water economy of the plant
by reducing transpiration;
mainly from the leaves; (3)
- b) Describe a special feature of Sorghum cells which enable the plant to tolerate high temperatures
enzymes resistant to higher temperatures than normal;
less easily inhibited / denatured; (2)
- c) Sorghum also has a reduced number of sunken stomata. Explain how these help Sorghum to survive in dry hot conditions.
most water loss from leaves (transpiration) is via open stomata;
less stomata reduce transpiration;
sunken stomata are protected from drying winds / low humidity;
sunken stomata in micro-climates of higher humidity;
reduces water loss by evaporation; (3)

Total 8

Question 6

Study the diagram below showing the pressure changes in the right hand side of the heart and, answer the following questions.

- a) i) Mark on the horizontal time axis the period during which the right ventricle is relaxing. (1)
ii) Describe the state of the tricuspid and pocket valves during this period.
tricuspid valves open;
pocket valves shut (2)
- b) Explain the negative pressure in the right atrium.
muscular walls relax after contracting;
small elastic recoil from fully contracted state; (2)
- c) i) From the graph deduce the highest (systolic) value for the blood pressure in the right ventricle. 25-30; (1)
ii) Explain why the pressure generated by the right ventricle is necessarily much lower than that generated by the left ventricle, which is about 120 mm Hg.
left ventricle requires higher pressure to pump blood around body;
right ventricle only pumps blood to lungs;
pressure needed as less resistance;
low pressure needed to prevent tissue fluid being exuded into alveoli; (3)
- d) With reference to the pressures indicated on the diagram explain the events in the right ventricle during one complete cardiac cycle.
starting from Time of 0 seconds on horizontal axis;
small rise in pressure as ventricle fills from atrium;
ventricles contract / ventricular systole;
pressure rises to its highest value at 0.3 seconds;
ventricles relax / ventricular diastole;
pressure drops accordingly;
negative pressure for about 0.3 seconds as ventricle walls recoil after contracting;
becomes positive as ventricles fill from atria;
cycle starts again; (6)

Total 14

Question 7

The simplified graph below shows the relationship between the rate of uptake of positively charged cations, negatively charged anions, and the rate of respiration.

- a) i) Briefly describe the relationship between the uptake of the ions and the rate of respiration.
uptake of cations increases with respiration rate;
uptake of anions shows no correlation with respiration rate (2)
- ii) Give a concise definition of active transport with regard to the uptake of ions by plants.
against the prevailing diffusion gradient;
uptake using energy from respiration in the form of ATP;
involves a membrane carrier; (3)
- iii) Identify from the graph which type of ion is more clearly taken up actively.
cations; (1)
- iv) Describe which part of the root is most involved in the uptake of inorganic ions from the soil
root hairs / region; (1)
- b) The diagram below represents the 'cation store' of the soil, which exists as the result of negatively charged clay particles holding positively charged cations.
- i) The different cations are held by attractions of different strengths, and are released and made available for uptake by the roots of plants after being displaced by hydrogen ions (H^+). Explain how respiration of root hair cells provides a supply of hydrogen ions (H^+).
aerobic respiration releases carbon dioxide;
carbon dioxide forms carbonic acid;
carbonic acid dissociates into hydrogen carbonate ions and hydrogen ions (protons); (3)
- ii) By reference to the diagram of the cation store explain why negatively charged anions such as nitrate ions must be continuously replenished in the soil.
negatively charged anions such as nitrate ions not held by negatively charged clay particles;
therefore free in soil solution;
easily leached in soil solution;
therefore must be continuously replenished in the soil naturally or artificially; (4)

Total 14

Exam Style Questions and mark schemes



1.1 Biological Molecules

1.2 Cells

1.3 Cell Transport

1.4 Exchange of Materials with the Environment

1.5 Enzymes

1.6 Digestion

Examination Style Question Papers

Biology only

Name: _____

I - Core Principles

Year Set 2

Time allowed 1 hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

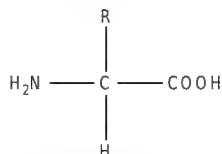
- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Section A

Answer all the questions in the spaces provided.

Question 1

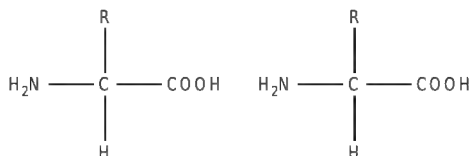
The structural formula of an amino acid is shown below.



- a) There are about 20 different types of amino acid to be found in proteins of living organisms, explain how the structural formula shown above applies to them all even though they are different.

(2)

- b) On the diagram below show how two amino acids can combine.



- c) Name the type of bond formed in the combination described in section b)

(2)

(1)

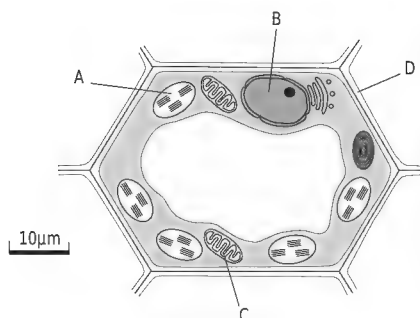
- d) Explain how only about 20 different types of amino acids can form the huge variety of proteins of found in living organisms.

(2)

Total 7

Question 2

Study the diagram below of a generalised plant cell as seen under the electron microscope and answer the following questions.



- a) Identify structures A to D

A _____
B _____
C _____
D _____

(4)

- b) If cells such as the one illustrated were treated in the appropriate way and subjected to differential ultracentrifugation, in what order would structures A, B, and C separate out.

(1)

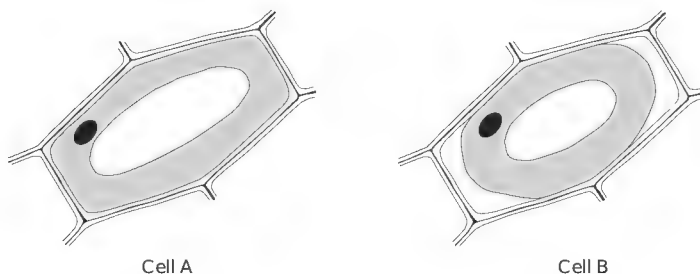
- c) Name three factors on which the rate of separation of A, B, and C, depends.

(3)

Total 8

Question 3

The diagrams below show two plant cells, each bathed in a different external solution.



- a i)** Identify the cell which is in a hypotonic solution (more dilute than the cell contents).

(1)

- ii)** Describe the appearance of the cell which is in a hypertonic solution (more concentrated than the cell contents).

(2)

- b)** Describe and explain what would happen to animal cells e.g. human red blood cells if they are placed in a hypotonic solution ?

(3)

Total 6

Question 4

a) Explain what is meant by the following terms with regard to the uptake of substances by cells:

i) facilitated diffusion

(2)

ii) active transport

(3)

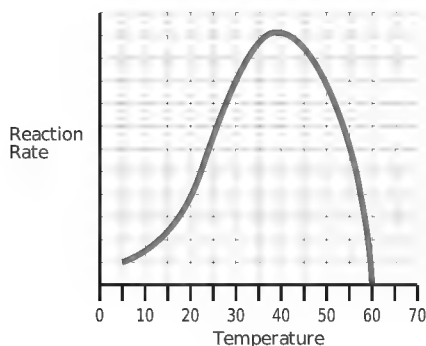
b) Explain why, when respiration is inhibited in cells bathed in pure water, substances, especially ions, are released from the cells.

(4)

Total 9

Question 5

The graph below shows the effect of changing temperature on the rate of an enzyme controlled reaction.



- a) i) Identify the optimum temperature for this enzyme.

(1)

- ii) Explain why the rate of reaction slows at temperatures below the optimum.

(2)

- iii) Explain why the rate of reaction slows at temperatures above the optimum..

(2)

- b) A graph of rate of enzyme reaction against pH would have a similar shape to the one above, but there is an important difference between the state of the enzyme at the lowest temperature and the state of the enzyme at the lowest pH. Explain this difference.

(2)

Total 7

Section B

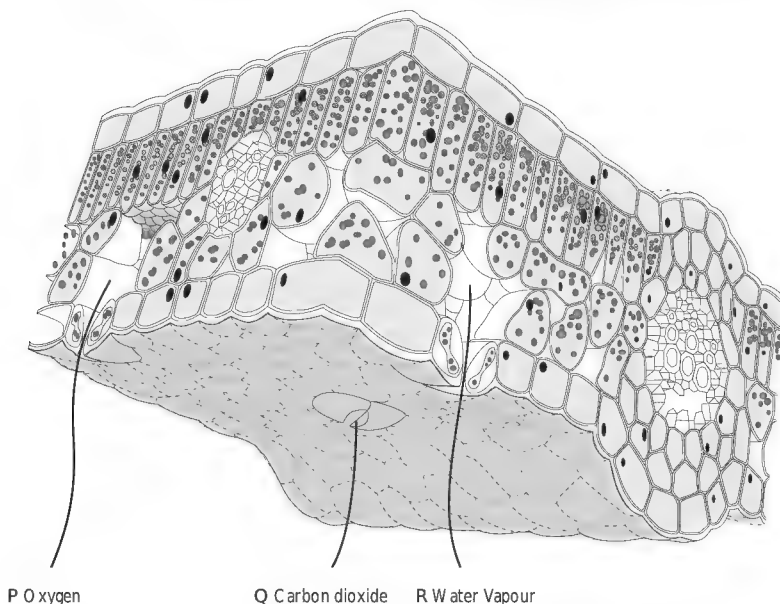
Answer all the questions in the spaces provided.

Answers should be written in continuous prose wherever appropriate.

Quality of written communication will be assessed in these answers.

Question 6

The diagram below is of a section through a typical leaf of a flowering plant (a mesophyte).

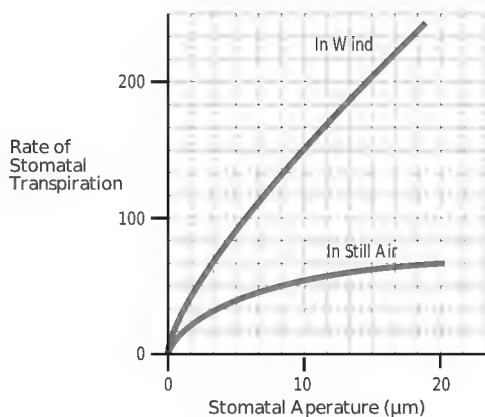


- a) i) On the diagram place arrow heads on the lines P, Q, and R passing through the stomata to show the overall direction of the diffusion of oxygen, carbon dioxide, and water respectively, at noon in conditions of average humidity. (3)
- ii) Indicate and name on the diagram a cell from which water is evaporating, before diffusing out of the leaf (1)
- b) With decreasing size of the stomata the passage of water vapour is restricted more than that of carbon dioxide. Explain the advantage of this to the plant in terms of gaseous exchanges with the environment.

(2)

Question continued...

- c) The graph below shows the relationship between the diameter of the stomatal aperture (hole) and the rate of loss of water vapour through the stomata at different conditions of external air movement.



Under which conditions do the stomata exert most control over the rate of loss of water vapour? Explain your answer.

(3)

- d) Describe the features of the leaf drawn on the previous page which provide a large surface area for gaseous exchange.

(6)

Total 15

Question 7

The drawing below is of an X-ray of the stomach and first part of the duodenum, after a barium meal (which shows up on X-rays).



a) Draw in and label on the diagram above the region of entry of the oesophagus into the stomach. (1)

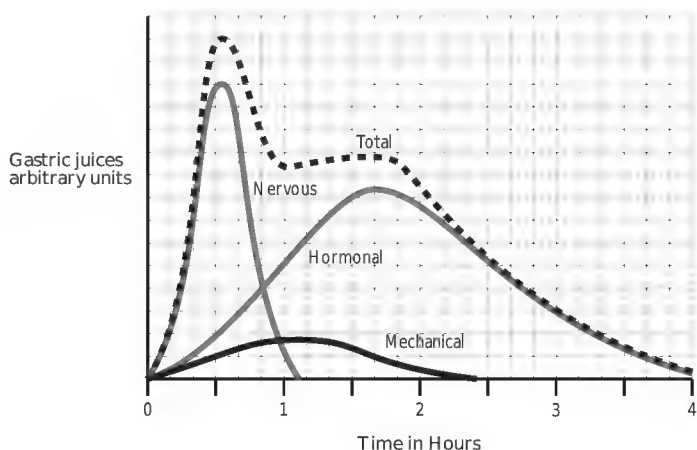
b) Describe and explain the appearance of the stomach in the diagram above. (4)

c) Describe and explain the appearance of the first part of the duodenum. (3)

d) Brunner's glands are a distinctive feature of the first part of the duodenum. Describe their function. (4)

Question continued...

- e) The graph below shows the phases of secretion of gastric juices after food enters the mouth.



- i) List the sequence in which the different stimuli have their major effect.

(1)

- ii) How long after food entering the mouth does the total gastric secretion reach its peak?

(1)

Total 14

Assessment Grid

Biology

I - Core Principles

Year Set 2

	Question Number							
Specification Section	1	2	3	4	5	6	7	Total
10.1 Biological molecules	7							7
10.2 Cells		8						8
10.3 Cell transport			6					6
10.4 Exchange of materials				9		15		24
10.5 Enzymes					7			7
10.6 Digestion							14	14
								Total 66

	Question Number							
Assessment Objective	1	2	3	4	5	6	7	Total
AO1								
Knowledge with Understanding	6	7	3	5	6	10	5	42
AO2								
Application/Analysis/Evaluation	1	1	3	4	1	5	9	24
								Total 66

Mark Schemes for Question Paper

Biology

I - Core Principles

Year Set 2

Instructions

; = 1 mark / = alternative response

Question I

The structural formula of an amino acid is shown below.

- a) There are about 20 different types of amino acid to be found in proteins of living origin, explain how the structural formula shown above applies to them all even though they are different.
the R group varies in structure;
providing the variation between them; (2)
- b) On the diagram below show how two amino acids can combine.
showing removal of elements of water;
formation of peptide bond CO-NH; (2)
- c) Name the type of bond formed in the combination described in section b)
condensation reaction; (1)
- d) Explain how only about 20 different types of amino acids can form the huge variety of proteins of living origin.
any combination of different amino acids;
any number of each type of amino acid;
resulting in a huge variety of different interactions between R groups etc;
leading to variety of secondary and tertiary structures; (2)

Total 7

Question 2

- a) Identify structures A to D

A = Chloroplast

B = Nucleus

C = Mitochondrion

D = Cell wall

(4)

- b) If cells such as the one illustrated were treated in the appropriate way and subjected to differential ultracentrifugation, in what order would structures A, B, and C separate out.

nucleus, chloroplast, mitochondrion;

(1)

- c) Name three factors on which their rate of separation depends

mass;

density compared to the suspending solution;

the gravitational force to which they are subjected;

(3)

Total 8

Question 3

The diagrams below show two plant cells, each bathed in a different external solution.

- a) i) Identify the cell which is in a hypotonic solution (more dilute than the cell contents.)
cell A; (1)
- ii) Describe the appearance of the cell which is in a hypertonic solution (more concentrated than the cell contents).
the cell membrane is withdrawn from the cell wall;
the central vacuole is smaller than in cell A; (2)
- iii) Describe and explain what would happen to animal cells e.g. human red blood cells if they are placed in a hypotonic solution?
water would enter by osmosis (endosmosis);
cells would increase in volume (swell);
cell membrane would be stretched;
and burst if water potential gradient high enough; (3)

Total 6

Question 4

a) Explain what is meant by the following terms with regard to the uptake of substances by cells:

i) facilitated diffusion

diffusion down a diffusion gradient;

from higher concentration to lower concentration;

speeded up/ made easier by the presence of special carriers in the membrane;

(2)

ii) active transport

uptake against the prevailing diffusion gradient;

from lower concentration to higher concentration;

involving carriers in cell membrane;

using energy from respiration / ATP;

(3)

b) Explain why, when respiration is inhibited in cells bathed in pure water, substances, especially ions, are released from the cells.

in pure water the diffusion gradient is from the more concentrated cell contents to the pure water;

this situation is maintained by active ion uptake;

using energy from respiration / ATP;

if respiration is inhibited active uptake is reduced and substances diffuse out of the cell into the surrounding water;

if respiration inhibited completely, membrane breaks down and all cell contents are released into the surrounding water;

(4)

Total 9

Question 5

The graph shows the effect of changing temperature on the rate of an enzyme controlled reaction.

- a) i) Identify the optimum temperature for this enzyme.

37°C;

(1)

- ii) Explain why the rate of reaction slows at temperatures below the optimum.

rate of collisions of reacting molecules temperature dependent;

the lower the temperature the lower the rate of collisions;

lowers the rate of all chemical reactions including enzyme catalysed;

(2)

- ii) Explain why the rate of reaction slows at temperatures above the optimum.

enzyme molecules progressively denatured / disrupted;

cannot form enzyme / substrate complexes;

rate of reaction slows despite increased rate of collision;

(2)

- b) A graph of rate of enzyme reaction against pH would have a similar shape as the one above, but there is an important difference between the state of the enzyme at the lowest temperature and the state of the enzyme at the lowest pH. Explain what this difference is.

at lowest temperature enzyme not denatured;

reversible;

at lowest pH enzyme denatured;

irreversible;

(2)

Total 7

Section B

Answer all the questions in the spaces provided.

Answers should be written in continuous prose wherever appropriate.

Quality of written communication will be assessed in these answers.

Question 6

The diagram below is of a section through a typical leaf of a flowering plant a mesophyte).

- a) i) On the diagram place arrow heads on the lines P, Q, and R passing through the stomata to show the overall direction of the diffusion of oxygen, carbon dioxide, and water respectively, at noon in conditions of average humidity. (3)
- ii) Indicate and name on the diagram a cell from which water is evaporating before diffusing out of the leaf (1)
- b) With decreasing diameter of the stomata the passage of water vapour is restricted more than that of carbon dioxide. Explain the advantage of this to the plant in terms of gaseous exchanges with the environment.
the main role of the stomata is for the uptake of carbon dioxide in the light;
but must also minimise loss of water vapour; (2)
- c) The graph below shows the relationship between the diameter of the stomatal aperture (hole) and the rate of loss of water vapour through the stomata at different conditions of external air movement.
Under what conditions do the stomata exert most control over the rate of loss of water vapour? Explain your answer.
in moving air;
as stomatal aperture rises so does rate of loss of water vapour;
in still air little increase in rate of loss of water vapour above 5 mm; (3)
- d) Describe the features of the leaf drawn on the previous page which provide a large surface area for gaseous exchange.
thin flat lamina blade);
with large surface area of leaf;
large intercellular air spaces between spongy mesophyll cells;
large intercellular air spaces between palisade mesophyll cells;
sub-stomatal cavities; (6)

Total 15

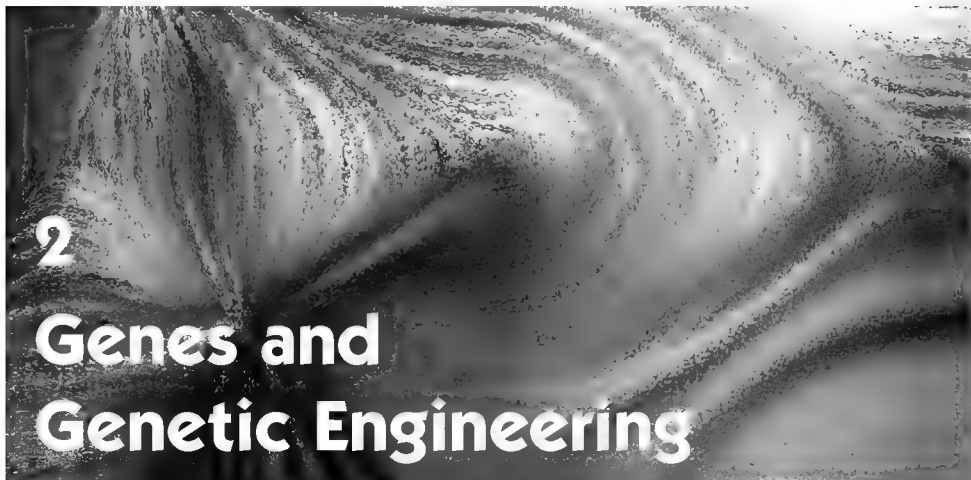
Question 7

The drawing below is of an X-ray of the stomach and first part of the duodenum after a barium meal (which shows up in X-rays).

- a) Draw in and label on the diagram above the region of entry of the oesophagus into the stomach. (1)
Tube from above diaphragm into stomach
- b) Describe and explain the appearance of the stomach. (4)
opaque barium meal shows up black;
clear area at top;
air and gastric secretions;
the 'J' shape demonstrates the 'hopper and mill' functions;
pyloric sphincter / constriction marks end of stomach / entry to duodenum;
- c) Describe and explain the appearance of the first part of the duodenum in the diagram above. (3)
small amounts of food chyle) entering from the stomach;
via the pyloric sphincter muscle;
constricted and expanded regions indicate;
peristaltic / wave-like contractions of circular and longitudinal muscle in wall;
moving the 'bolus' along the duodenum;
- d) Brunner's glands are a distinctive feature of the first part of the duodenum. Describe their function. (4)
secrete an alkaline juice;
rich in mucus;
to protect lining of the first part of the duodenum;
against damage by acid / neutralises acid from the stomach;
- e) The graph below shows the phases of secretion of gastric juices after food enters the mouth. (1)
i) List the sequence in which the different stimuli have their major effect. (1)
nervous, mechanical, hormonal
- ii) How long after food entering the mouth does the total gastric secretion reach its peak? (1)
about 30 minutes;

Total 14

Exam style question papers



2.1 The Genetic Code

2.2 The Cell Cycle

2.3 Sexual Reproduction

2.4 Application of Gene Technology

Examination Style Question Papers

Name: _____

Biology

2 - Genes and Genetic Engineering Year Set 2

Time allowed 1 hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Question 1

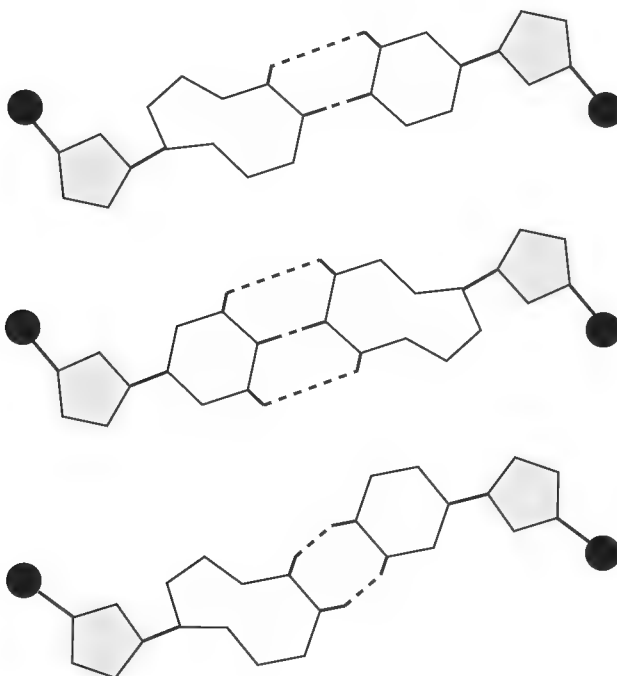
- a) List the components common to all DNA nucleotides.

(1)

- b) Describe the way in which the four types of DNA nucleotide differ from each other.

(2)

- c) The diagram below represents base pairings and hydrogen bonding between pairs of bases in the DNA double helix, fill in the names of the appropriate bases in the boxes



(2)

Total 5

Question 2

- a) Gene mutations may occur naturally at random. List three environmental agents that increase the rate at which natural gene mutations occur.

(3)

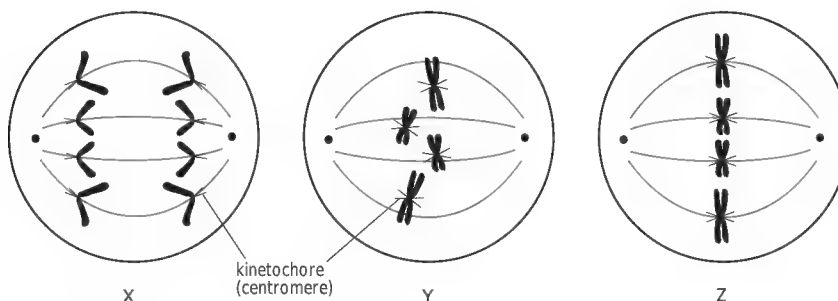
- b) Identify the types of gene mutation illustrated below, and reading from the left underline the changed sequences of bases on the strand of DNA.

Normal strand of DNA	ATT CGC CGA ATT	
i) Gene mutation	ATT CGG CCG AAT T	(1)
ii) Gene mutation	ATC GCC GAA TT	(1)
iii) Gene mutation	ATT CGT CGA ATT	(1)

Total 6

Question 3

Study the diagrams below of different stages in mitosis and answer the following questions.



- a) i) Write the letters in the correct sequence that these stages occur in mitosis, and name each stage.

(2)

- ii) Describe what is occurring with respect to the chromosomes / chromatids in each of the stages shown in diagrams Y and Z.

(2)

- (b) Explain the shape of the chromosomes in diagram X.

(3)

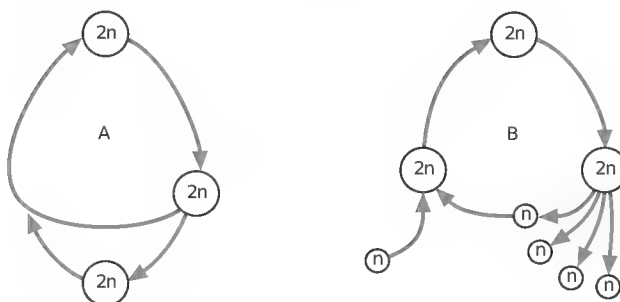
Total 6

Question 4

The diagram below represents the amount of DNA present in the nuclei of cells during the different cell cycles of an organism, where:

$2n$ = normal diploid amount of DNA, and

n = half the diploid amount i.e. the haploid amount.



- a) Identify which cell cycle involves meiosis, and explain your choice.

(2)

- b) Label on the diagram above two different gametes (sex cells), and indicate with an X on the diagram where fertilisation takes place.

(1)

- c) Give two major differences between male and female gametes as seen in most living organisms.

(2)

Total 5

Question 5

- a) Describe three ways in which mitosis is involved in the life of a multicellular organism.

(3)

- b) By reference to a named example describe how mitosis is involved in the life of a unicellular organism.

(2)

- c) Colchicine is a chemical which inhibits nuclear spindle fibre formation. Describe in terms of chromosome number the effect of colchicine on a mitotically dividing nucleus.

(2)

Total 7

Question 6

The table below summarises the effects of two antibiotics on genetically modified (engineered) bacteria, which had been exposed to plasmids containing either the two genes for resistance to the two antibiotics in the table, or the gene for resistance to one antibiotic and the gene for human insulin.

Bacteria Resistant to antibiotics	Ampicillin	Tetracycline
A	No	No
B	Yes	Yes
C	Yes	No

- a) i) Describe the nature of plasmids.

(1)

- ii) Describe and explain the treatment to which bacteria can be exposed that encourages the uptake of plasmids in such an experiment.

(2)

- iii) From the results shown in the table above describe the state of bacteria A, B, and C in terms of which plasmids they have taken up.

A

B

C

(1)

- b) Given the results in the table, briefly describe how bacteria carrying the gene for human insulin could be isolated from a mixed culture.

(3)

Total 7

SECTION B

Question 7

Gene therapy in which engineered / modified genes are produced to provide a properly working version of a damaged / mutated gene in a patient, poses the major problem of delivery to the site where the modified gene will be most effective.

- a) i) In accounts of genetic engineering the use of the singular term '**the** correctly working **gene**' could mislead people not familiar with genetics. Explain the situation with regard to cells, nuclei, chromosomes and genes to give a clearer and more accurate version of introducing 'a gene' into a patient.

(4)

- ii) Describe the advantages of introducing 'a genetically modified gene' into stem cells rather than normal body cells.

(3)

- b) Several gene delivery systems can be used to introduce engineered genes into a patient, including aerosols, liposomes, ballistic DNA injection, viruses and the use of extracted cells.

- i) Describe a condition in which the use of aerosol delivery could be used, and explain why it is particularly suitable in this case.

(3)

- ii) Describe a major problem with the use of viruses as vectors of engineered genes.

(2)

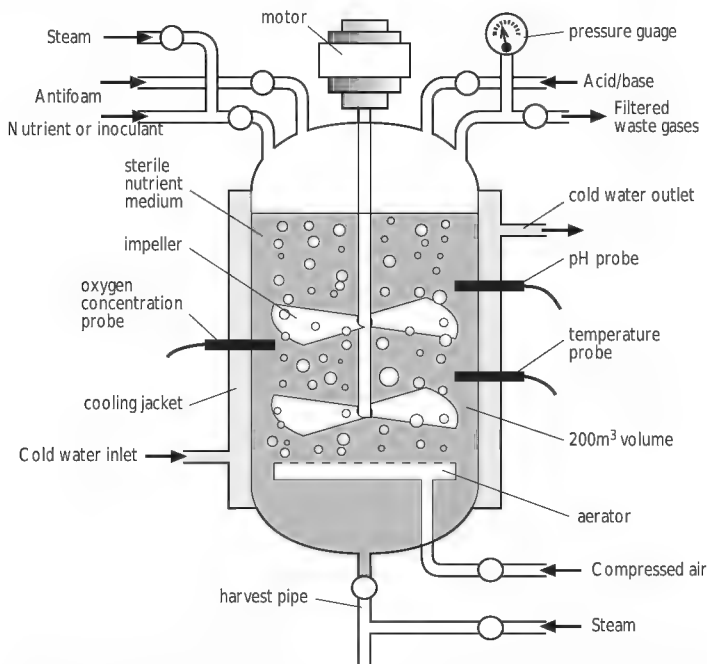
- c) Describe which cells should be targeted if an attempt was to be made to introduce engineered genes, that could be inherited by any offspring of the patient. Explain your choice.

(3)

Total 15

Question 8

In genetic engineering, genetically modified bacteria can be cultured on a large scale in industrial fermenters or bioreactors. Study the simplified diagram of a bioreactor below and answer the following questions.



a) The bioreactor has many inputs and outputs, briefly describe the role of the following.

i) Compressed air.

(2)

ii) Acid/Base

(2)

iii) Steam

(1)

iv) Antifoam

(2)

Question continued...

-
- b)** Explain why contaminant bacteria, if they get into the culture, generally outcompete the engineered bacteria for nutrients and oxygen etc.

(3)

- c)** Describe two reasons why good mixing is necessary in an operating bioreactor.

(2)

- d)** Explain why the scaling up of laboratory process to a large scale industrial process poses special problems of heat production and heat loss.

(3)

Total 15

Assessment Grid

Biology

Unit
ear Set 2

	Question Number								
Specification Section	1	2	3	4	5	6	7	8	Total
11.1 Genetic code	5	6					15		26
11.2 Cell cycle			7	5					12
11.3 Sexual reproduction					7				7
11.4 Gene Technology						7			15
Total 66									

	Question Number								
Assessment Objective	1	2	3	4	5	6	7	8	Total
AO1									
Knowledge with Understanding	5	6	3	2	5	3	7	9	40
AO2									
Application/Analysis/Evaluation			4	3	2	4	8	6	26
Total 66									

Mark Schemes for Question Paper

Biology

2 - Genes and Genetic Engineering Year Set 2

Instructions

; = 1 mark / = alternative response

Section A

Question 1

- a) List the components common to all DNA nucleotides.
ribose sugar / deoxyribose sugar;
phosphate group;
nitrogenous base; (1)
- b) Describe the way in which the four types of DNA nucleotide differ from each other.
four different bases;
adenine, thymine, cytosine, guanine; (2)
- c) The diagram below represents base pairings and hydrogen bonding between pairs of bases in the DNA double helix, fill in the names of the appropriate bases in the boxes. (2)

Total 5

Question 2

- a) Gene mutations may occur naturally at random. List three classes of environmental agents that increase the rate at which natural mutations occur.

high energy radiation;

high energy particles;

mutagenic chemicals

(3)

- b) Identify the types of gene mutation illustrated below, and reading from the left underline the point of the change to the bases on the strand of DNA.

Normal strand of DNA

ATT CGC CGA ATT

- i) Gene mutation

ATT CGG CCG AAT T

(1)

- ii) Gene mutation

ATC GCC GAA TT

(1)

- iii) Gene mutation

ATT CGT CGA ATT

(1)

(3)

Total 6

Question 3

Study the diagrams below of different stages in mitosis and answer the following questions.

- a) i) Write the letters in the correct sequence that these stages occur in mitosis.

Y Z X

(1)

- ii) Describe what is occurring with respect to the chromosomes / chromatids in each of the stages shown in diagrams Y and Z.

Y - the chromosomes (each of two chromatids) are just formed and moving towards the equator of the nuclear spindle apparatus;

(1)

Z - the chromosomes (each of two chromatids) are arranged on the equator by means of the kinetochore centromere) attached to fibres of the spindle apparatus;

(1)

- b) Explain the shape of the chromosomes in diagram X.

the kinetochores centromeres) are the centres of movement of the chromosomes;

they lead the way along the spindle fibres towards the centrioles at the poles of the spindle;

the 'arms' of the chromosomes trail behind;

(3)

Total 6

Question 4

The diagram below represents the amount of DNA present in the nuclei of cells during the different cell cycles of an organism, where:

$2N$ = normal diploid amount of DNA, and

N = half the diploid amount i.e. the haploid amount.

- a) Identify which cell cycle involves Meiosis, and explain your choice.
B;
the amount of DNA in the daughter nuclei is halved;
from the diploid quantity to the haploid quantity; (2)
- b) Label on the diagram above two different gametes (sex cells), and indicate with an X on the diagram where fertilisation takes place. (1)
- c) Give two major differences between male and female gametes as seen in most living organisms.
female gametes larger than male;
larger number of male gametes produced than female;
male gametes mobile, female gametes static; (2)

Total 5

Question 5

- a) Describe three ways in which mitosis is involved in the life of a multicellular organism.
- growth;
repair of damaged tissue;
asexual reproduction; (3)
- b) By reference to a named example describe how mitosis is involved in the life of a unicellular organism.
- asexual reproduction by binary fission / sporulation / etc;
e.g. yeast / amoeba / bacteria / etc; (2)
- c) Colchicine is a chemical which inhibits nuclear spindle fibre formation. Describe in terms of chromosome number the effect of colchicine on a mitotically dividing nucleus.
- chromatids will not separate;
 $2n \rightarrow 4n$; (2)

Total 7

Question 6

The table below summarises the effects of two antibiotics on genetically modified (engineered) bacteria, which had been exposed to plasmids containing either the two genes for resistance to the two antibiotics in the table, or the gene for resistance to one antibiotic and the gene for human insulin.

- a) i) Describe the nature of plasmids.
circular / ring like DNA in bacterial cytoplasm; (1)
- ii) Describe and explain the treatment to which bacteria can be exposed that encourages the uptake of plasmids in such an experiment.
ice-cold calcium chloride;
after 15 minutes in ice 90 seconds at 42°C
Agitation; (2)
- iii) From the results shown in the table above describe the state of bacteria A, B, and C in terms of which plasmids they have taken up.
A none taken up
B unaltered plasmids taken up
C plasmids with gene for human insulin taken up; (1)
- b) Given the results in the table, briefly describe how bacteria carrying the gene for human insulin could be isolated from a mixed culture of A,B and C.
plate out on agar plates containing ampicillin;
B and C will grow;
replica plate onto agar plates containing both antibiotics;
only B colonies will grow on this;
return to previous plate and take sample from colonies) of C; (3)

Total 7

SECTION B

Question 7

Gene therapy in which engineered / modified genes are produced to provide a properly working version of a damaged / mutated gene in a patient poses the major problem of delivery to the site where the modified gene will be most effective.

- a) i) In accounts of genetic engineering the use of the singular term '**the** correctly working **gene**' could mislead people not familiar with genetics. Explain the situation with regard to cells, nuclei, chromosomes and genes, to give a clearer and more accurate version of introducing 'a gene' into a patient.

the nuclei of virtually all cells in the body are genetically identical;

with a full diploid number of chromosomes (totipotent);

the same gene is found at the same position (locus) on all copies of that particular chromosome;

so the phrase 'the or a gene' refers to a type of gene not its frequency; (4)

- ii) Describe the advantages of introducing 'a genetically modified gene' into stem cells rather than normal body cells.

most body cells fully differentiated and incapable of further division;

stem cells are undifferentiated and can divide and differentiate into all other cell types;

an introduced modified gene would be multiplied and incorporated accordingly; (3)

- b) Several gene delivery systems can be used to introduce engineered genes into a patient, including aerosols, liposomes, ballistic DNA injection, viruses and the use of extracted cells.

- i) Describe a condition in which the use of aerosol delivery could be used, and explain why it is particularly suitable in this case.

cystic fibrosis caused by a deficiency in a cell surface membrane protein;

resulting in congested respiratory system (amongst other symptoms);

aerosol delivery gets in direct contact with tissue involved with major symptom; (3)

- ii) Describe two problems associated with the use of viruses as vectors of engineered genes.

virus introduces its own genes into host which may be harmful;

virus stimulates immune system to destroy viruses; (2)

- c) Describe which cells should be targeted if an attempt was to be made to introduce engineered genes, that could be inherited by any offspring of the patient. Explain your choice.

germ line cells which produce gametes by meiosis;

the only cells with direct physical continuity with next generation;

therefore introduced gene(s) could be passed on in the DNA; (3)

Total 15

Question 8

In genetic engineering, bacteria containing the transferred gene can be cultured on a large scale in industrial fermenters or bioreactors. Study the simplified diagram of a bioreactor below and answer the following questions.

- a) The bioreactor has many inputs and outputs, briefly describe the role of the following.
- i) Compressed air.
supply of air containing oxygen;
for respiration of aerobic bacteria (2)
 - ii) Acid/Base
to adjust the pH to optimum conditions for enzyme activity of microorganisms;
as it fluctuates as a result of their metabolism; (2)
 - iii) Steam
sterilisation between batches; (1)
 - iv) Antifoam
prevents the accumulation of excess foam as a result of gases released in fermentation;
which would otherwise fill system and interfere with flow processes; (2)
- b) Explain why contaminant bacteria, if they get into the culture, generally outcompete the engineered bacteria for nutrients and oxygen etc.
engineered bacteria have a considerable part of their metabolism diverted to production of 'foreign' protein;
depresses rates of growth and reproduction;
contaminant 'wild' type bacteria direct all their resources to their own growth and reproduction; (3)
- c) Describe two reasons why good mixing is necessary in an operating bioreactor.
to ensure all necessary supplies are uniformly and best dispersed;
to ensure even distribution of microorganisms to best access supplies / dissipate heat; (2)
- d) Explain why the scaling up of laboratory process to a large scale industrial process poses special problems of heat production and heat loss.
with increasing size volume increases by the cube and the surface area by the square;
volume of microorganisms generate heat;
which must be dissipated over the surface; (3)

Total 15

Exam style question papers



3.1 Transport Systems

3.2 The Control of Breathing and Heartbeat

3.3 Energy and Exercise

3.4 The Transport of Substances in Plants

Examination Style Question Papers

Name: _____

Biology

3 - Physiology & Transport

Year Set 2

Time allowed 1 hour 15 minutes

Instructions:

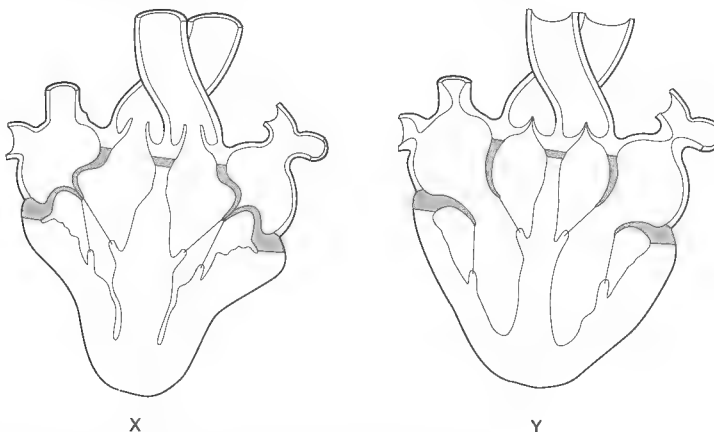
- Answer all the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Question 1

Study the diagrams below showing the state of the heart at two stages in the cardiac cycle, and answer the following questions.



- a) i) Identify the diagram in which the ventricles are relaxed.

(1)

- ii) By means of a cross on one of the diagrams identify the chamber in which the pressure is highest.

(1)

- iii) By means of arrows on both of the diagrams above indicate the direction of the blood flow into and out of the heart.

(1)

- b) Describe what causes the pocket valves to shut at the point in the cardiac cycle illustrated by diagram Y.

(3)

- c) i) Account for the difference between the position of the atrio-ventricular valves in diagrams X and Y.

(2)

- ii) Describe how are these valves prevented from opening in the wrong direction.

(1)

Total 9

Question 2

a) Give brief descriptive definitions of each of the following:

i) plasma

(2)

ii) tissue fluid

(2)

iii) lymph

(2)

b) i) Name one difference between a blood capillary and a lymphatic capillary.

(1)

ii) Describe how the lymph is moved through the lymphatic system and back to the circulation.

(3)

Total 10

Question 3

- a) Name and describe the location of the centre for the nervous control of breathing in humans.

(1)

- b) This control centre receives sensory nervous input from receptors in various parts of the body.

- i) Name and describe the location of such a receptor which is sensitive to mechanical stimuli.

(1)

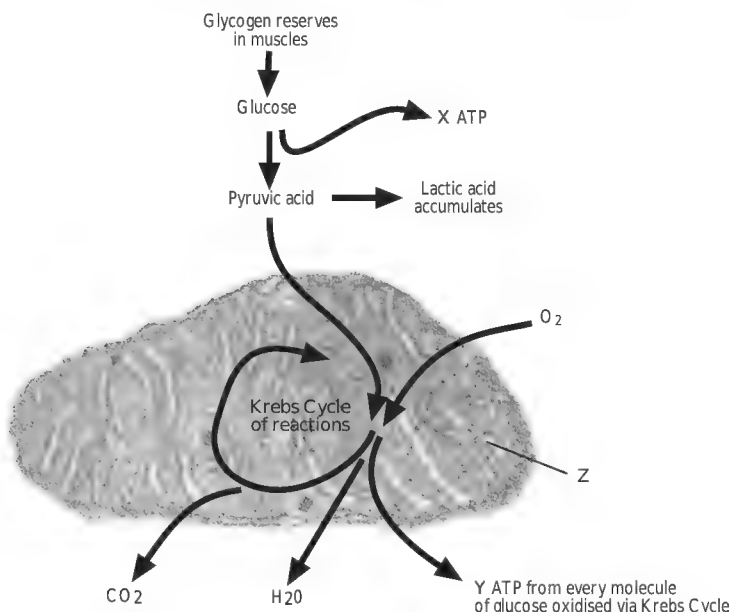
- ii) Name and describe the location of such a receptor which is sensitive to chemical stimuli.

(1)

Total 3

Question 4

Study the simplified diagram below of the metabolic pathways involved in energy release from glycogen and glucose, and answer the following questions.



- a) State the number of ATP molecules synthesised (per molecule of glucose oxidised) at points X and Y.

(2)

- b) State where in the cell the stages up to point X occur.

(1)

- c) Describe the activity of the body and the conditions under which the pathway leading to the production of lactic acid operates.

(3)

- d) On the diagram label the much magnified structure Z.

(1)

- e) Add a labelled line 'F' to the diagram showing where the oxidation of fats would link in with the other pathways.

(1)

Total 8

Question 5

- a) Give two structural differences between phloem tissue and xylem tissue that are relevant to their functions.

(i) _____

(ii) _____

(2)

- b) (i) State the function of the phloem.

(2)

- ii) Describe one piece of evidence that supports the function of phloem you have given.

(2)

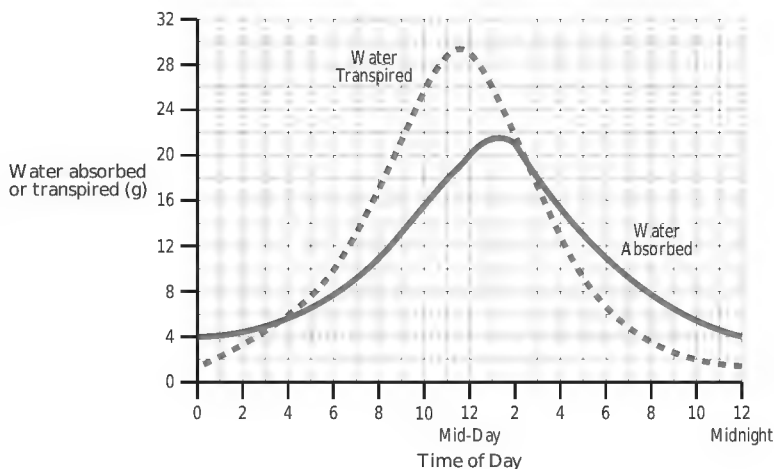
- c) If a length of stem of a living plant is killed by heating in a 'steam jacket' applied to that region, translocation stops but transpiration continues. Explain these observations with regard to the nature of phloem and xylem.

(2)

Total 8

Question 6

The graph below shows the relationship between the rate of water uptake by the roots of a plant and its transpiration rate over a 24 hour period, under natural conditions on a summer's day.



- a) i) Identify the times when transpiration and water uptake reach their peaks.

(1)

- ii) Give one environmental factor other than temperature which reaches a peak near the time of maximum transpiration.

(1)

- iii) Explain the cause and effect relationship between the environmental factor peaking and the maximum transpiration rate.

(4)

- b) Describe the environmental conditions under which transpiration could continue at a high level at least for a short period) but water uptake is reduced to a minimum.

(2)

Question continued...

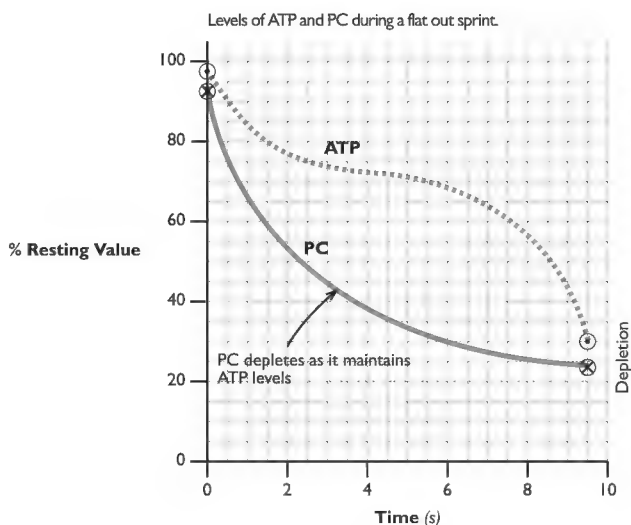
-
- c) With reference to the graph describe how the curves help to explain the cohesion-tension mechanism of moving water through the xylem.

(6)

Total 14

Question 7

Study the graph of the rate of usage of ATP and phosphocreatine (PC) in a flat-out sprint run and answer the following questions.



- a) With reference to the graph, describe and explain why 100 metre runners start slowing down after about 6 seconds.

(3)

- b) Explain why the curve for phosphocreatine (creatine phosphate) falls in the way that it does.

(3)

- c) The amount of ADP / ATP in the body is largely fixed and cannot be increased by training or supplementation of the diet, whereas the amount of CP can be increased in these ways. Explain how increasing the amount of creatine in the diet can increase the reserves of CP in the body and describe what type of training would encourage this.

(2)

Question continued...

-
- d) i)** Explain how the supply of energy for muscle contraction represented in the graph can be rapid enough for a flat out sprint.

(4)

- ii)** Once the CP and ATP is depleted, describe the metabolic pathway which is the next immediate source of energy for maximally contracting muscles.

(2)

Total 14

Assessment Grid

Biology

3 - Physiology & Transport
Year Set 2

	Question Number							
Specification Section	1	2	3	4	5	6	7	Total
12.1 Transport systems	9	10						19
12.2 Breathing and heartbeat			3					3
12.3 Energy and exercise				8			14	22
12.4 Transport in plants					8	14		22
								Total 66

	Question Number							
Assessment Objective	1	2	3	4	5	6	7	Total
AO1								
Knowledge with Understanding	7	10	1	4	6	8	8	44
AO2								
Application/Analysis/Evaluation	2		2	4	2	6	6	22
								Total 66

Mark Schemes for Question Paper

Biology

3 - Physiology & Transport Year Set 2

Instructions

; = 1 mark / = alternative response

Question 1

Study the diagrams below showing the state of the heart at two stages in the cardiac cycle, and answer the following questions.

Diagrams of heart at ventricular diastole (X) and systole (Y)

- a) i) Identify the diagram in which the ventricles are relaxed. (1)
Y
- ii) By means of a cross on one of the diagrams identify the chamber in which the pressure is highest. (1)
Cross correctly identifying the left ventricle in diagram X
- iii) By means of arrows on both of the diagrams above indicate the direction of blood flow into and out of the heart. (1)
Direction of flow of blood correctly shown on both diagrams.
- b) Describe what causes the pocket valves to shut at the point in the cardiac cycle illustrated by diagram Y (3)
by blood falling back down the blood vessels exiting the right and left ventricles (aorta and pulmonary arteries);
as a result of the lower pressure in the empty ventricles;
blood fills 'pockets';
'pockets' block and shut the blood vessels;
- c) (i) Account for the difference between the position of the atrio-ventricular valves in diagrams X and Y. (2)
in Y the ventricles are relaxed and blood has been forced down from the atria opening the valves;
in X the ventricles have contracted forcing blood up into the aorta and pulmonary artery pushing the valves shut;
- (ii) Describe how are these valves prevented from opening in the wrong direction. (1)
prevented from opening the wrong way by tendon cords becoming taut;
aided by muscles of ventricular wall exerting a downward pull on tendon cords;

Total 15

Question 2

a) Give brief descriptive definitions of each of the following:

i) plasma

the fluid part of the blood;
dilute clear - yellow aqueous solution;
containing soluble substances e.g. inorganic ions;
containing proteins;
eg anti-bodies / enzymes

(2)

ii) tissue fluid

that part of the plasma exuded through the capillary walls;
as a result of blood pressure / water potential gradient;
minus plasma proteins;
bathes tissues directly;

(2)

iii) lymph

tissue fluid absorbed into lymphatic capillaries;
contains lymphocytes;
fatty globules after a meal;

(2)

b) i) Name one difference between a blood capillary and a lymphatic capillary.

lymphatic capillary blind ending;

(1)

ii) Describe how the lymph is moved through the lymphatic system and back to the circulation.

contractions of surrounding skeletal musculature;
massage thin walled lymph vessels;
one way valves ensure one way flow;

(3)

Total 10

Question 3

- a) Name and describe the location of the centre for the nervous control of breathing in humans.
respiratory centre / inspiratory and expiratory centres in the medulla of the brain. (1)
- b) This control centre receives sensory nervous input from receptors in various parts of the body.
- i) Name and describe the location of such a receptor which is sensitive to mechanical stimuli.
stretch receptors in the alveoli; (1)
- ii) Name and describe the location of such a receptor which is sensitive to chemical stimuli.
chemoreceptor in carotid / aortic body; (1)

Total 3

Question 4

Study the simplified diagram below of the metabolic pathways involved in energy release from glycogen and glucose, and answer the following questions.

- a) State the number of ATP molecules synthesised (per molecule of glucose oxidised) at points X and Y.
X - 2 ATP;
Y - 31 or any similar number (unresolved dispute); (2)
- b) State where in the cell stages up to point X occur.
cytoplasm / outside of the mitochondria; (1)
- c) Describe the activity of the body and the conditions under which the pathway leading to the production of lactic acid operates.
intense anaerobic effort;
when insufficient oxygen supplied to the cells / muscle fibres;
when insufficient oxygen supplied to the mitochondria; (3)
- d) Label the much magnified structure Z.
mitochondrion; (1)
- e) Add a labelled line 'F' to the diagram showing where the oxidation of fats would link in to the other pathways.
between pyruvic acid and membrane of mitochondrion; (1)

Total 8

Question 5

- a) List two structural differences between phloem tissue and xylem tissue that are relevant to their functions.

Phloem	Xylem	
cytoplasmic contents	empty cell lumen;	
cellulose cell walls	lignified cell walls;	
sieve plates	no end walls;	(2)

- b) (i) State the function of the phloem.

transport / translocation of organic substances;
from 'sources' to 'sinks' (2)

- ii) Describe one piece of evidence that supports the function of phloem you have given.

aphid stylets penetrate phloem;
exude sucrose solution;
or
isotopically labelled carbon compounds;
mainly in phloem; (2)

- c) If a length of stem of a living plant is killed by heating in a 'steam jacket' applied to that region, translocation stops but transpiration continues. Explain these observations with regard to the nature of phloem and xylem.

phloem must be living for translocation to occur;
xylem is a dead tissue unaffected by heat; (2)

Total 8

Question 6

The graph below shows the relationship between the rate of water uptake by the roots of a plant and the transpiration rate over a 24 hour period under natural conditions on a summer's day.

- a) i) Identify the times when transpiration and water uptake reach their peaks.
transpiration around 12 and water uptake around 2 pm; (1)
- ii) Give one environmental factor other than temperature which reaches a peak near the time of maximum transpiration.
light intensity; (1)
- iii) Explain the cause and effect relationship between the environmental factor peaking and the maximum transpiration rate.
transpiration occurs mainly via the stomata;
stomata open in the light;
therefore the period of greatest stomatal opening and greatest transpiration;
is period of maximum light intensity (4)
- b) Describe the environmental conditions under which transpiration could continue at a high level at least for a short period) but water uptake is reduced to a minimum.
dry hot conditions;
soil water at a minimum; (2)
- c) With reference to the graph describe how the curves help to explain the cohesion-tension mechanism of moving water through the xylem.
peak of transpiration occurs before that of water absorption;
ie water loss greater than water gain;
therefore water in xylem under negative tension;
as water pulled up by cohesion-tension;
rather than pushed up by positive root pressure;
in which case peaks would be in reverse order; (6)

Total 14

Question 7

Study the graph of the rate of usage of ATP and phosphocreatine (PC) in a flat-out sprint run and answer the following questions.

- a) With reference to the graph, describe and explain why 100 metre runners start slowing down after about 6 seconds.

final decline / depletion of ATP starts around that point;

ATP is the immediate energy source for muscle contraction;

therefore energy supply to contracting muscles decreases; (3)

- b) Explain why the curve for phosphocreatine (creatine phosphate) falls in the way that it does.

ATP the only form in which energy can be directly used by the body;

phosphocreatine is immediate source of regeneration of ATP;

therefore as ATP depleted so is PC; (3)

- c) The amount of ADP / ATP in the body is largely fixed and cannot be increased by training or supplementation of the diet, whereas the amount of CP can be increased in these ways. Explain how increasing the amount of creatine in the diet can increase the reserves of CP in the body, and describe what training would reinforce this.

increased creatine combines with phosphate normally present in diet;

training which depletes and allows regeneration of stores of phosphocreatine ie repeat sprints; (2)

- d) i) Explain why the supply of energy for muscle contraction represented in the graph can be rapid enough for a flat out sprint.

ATP and CP are both present in the muscle fibres;

this phosphagen store is available for immediate use;

no metabolic pathways immediately involved;

no transport pathways immediately involved; (4)

- ii) Once the CP and ATP is depleted, describe the metabolic pathway which is the next immediate source of energy for maximally contracting muscles.

lactic / anaerobic pathway;

breakdown of glucose in the absence of oxygen; (2)

Total 14

Exam Style Questions and mark schemes



1.1 Biological Molecules

1.2 Cells

1.3 Cell Transport

1.4 Exchange of Materials with the Environment

1.5 Enzymes

1.6 Digestion

Examination Style Question Papers

Name: _____

Biology

I - Core Principles

Year Set 3

Time allowed | hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Section A

Answer all the questions in the spaces provided.

Question 1

A solution of unknown composition is tested with Benedict's solution, and a positive result is obtained.

- a) Describe a positive result with the Benedict's test.

(2)

- a) Explain why it would be wrong to deduce that the positive Benedict's test indicates that glucose is present.

(3)

- c) Explain why it would not be correct to deduce from a positive Benedict's test that there were no disaccharides present in the solution.

(3)

Total 8

Question 2

- a) Give the names of two proteins in the body.

(1)

- b) Describe the biuret test for proteins, and the appearance of a positive result.

(2)

- c) Explain how the shape of the three dimensional tertiary structure of an enzyme is essential for its role as a biological catalyst.

(3)

Total 6

Question 3

- a) Plant and animal cells are both described as eukaryotic cells, describe the main features of eukaryotic cells.

(4)

- b) Name a type of organism which has a prokaryotic cell. State two differences between this prokaryotic cell and a eukaryotic animal cell.

(3)

Total 7

Question 4

- a) In the table below list three differences between light (optical) and electron microscopes

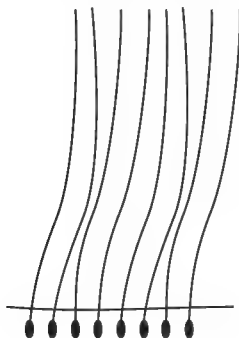
Light microscope	Electron microscope

(3)

- b) Describe the main difference between the mode of functioning of the transmission and scanning electron microscopes.

(2)

- c) Drawing X represents cilia. Drawing Y represents the appearance of a section of cilia.



X



Y

Interpret and explain the difference in appearance between the two cell diagrams.

(3)

Total 8

Question 5

- a) i) What is the water potential of pure water under normal conditions?

_____ (1)

- ii) Explain why alternatives to the term 'concentration of water' have been devised.

_____ (2)

- iii) Name a factor to which the rate of osmosis is proportional.

_____ (1)

- b) If a plant cell is placed in pure water, it eventually becomes fully turgid. The cellulose cell wall can expand no further, and so no more water enters the cell. Explain this process in terms of the effect of pressure on water potential.

_____ (4)

Total 8

Section B

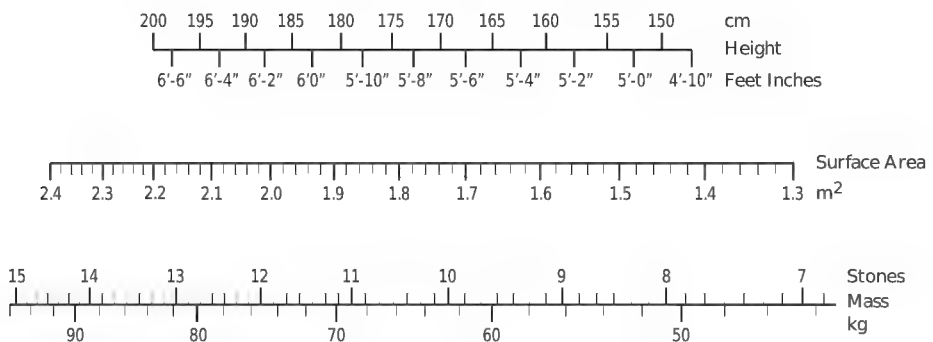
Answer all the questions in the spaces provided.

Answers should be written in continuous prose wherever appropriate.

Quality of written communication will be assessed in these answers.

Question 6

The relationship between the size of an organism or structure, and the surface area to volume ratio is significant in the exchange of substances and heat. The nomogram below enables the surface area of an individual to be estimated from their height and weight.



a) Estimate the surface area of two individuals X and Y:

i) X with a height of 195 cm and a weight of 71 kg,

_____ (1)

ii) Y with a height of 165 cm and a weight of 72 kg.

_____ (1)

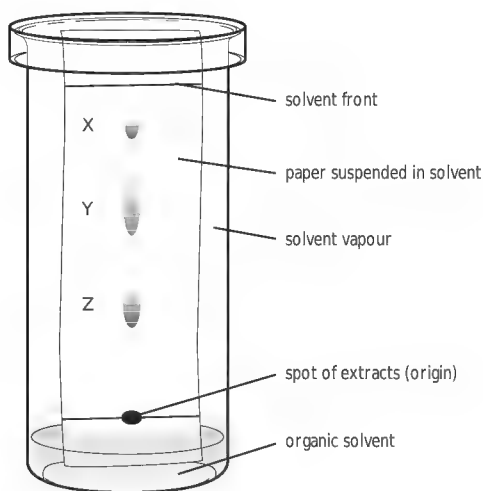
Question continued...

- [illegible]

Total 15

Question 7

There are many different chromatography techniques, but the original one is paper chromatography. The initial set up for a simple separation using paper chromatography is shown below.



- a) Calculate the Rf value for substance X. Show your workings.

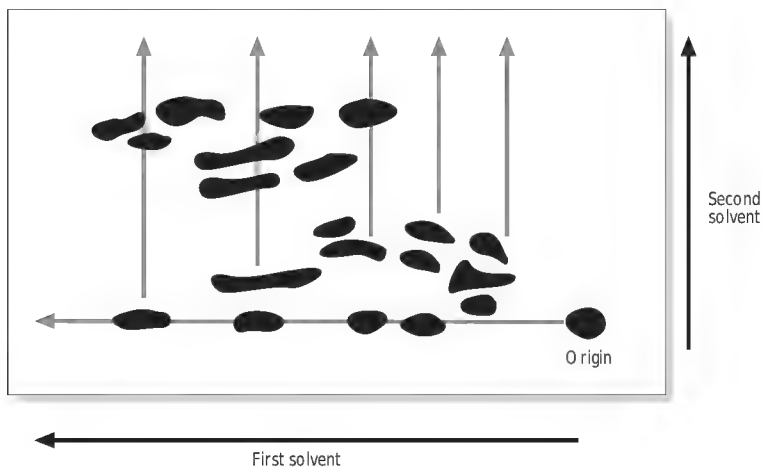
(4)

- b) Describe what extra treatment of the paper chromatogram would be necessary if the substance under investigation was not a pigment.

(2)

Question continued...

- c) In two way chromatography a chromatogram which has undergone one way chromatography as described previously, is turned on its side, and the process is repeated using a different solvent as shown below.

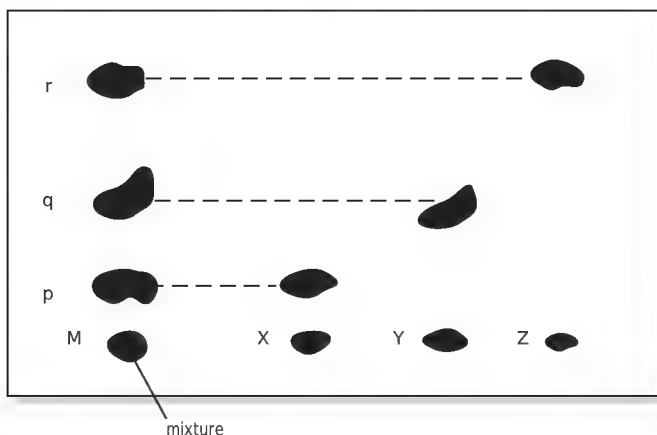


- i) Describe the purpose of two way chromatography, and explain why a solvent different from that used in the first run must be used in the second run.

(3)

Question continued...

- ii) In addition to the use of R_f values, substances on a chromatogram may be identified by comparison with the positions of known substances as shown below.



In the example shown on the diagrams above, identify substance q.

(1)

- d) Explain the principles upon which paper chromatography is based.

(3)

- e) Explain why touching the chromatography paper before starting a run to investigate a sample of amino acids would invalidate (undermine) the results.

(2)

Total 15

Assessment Grid

Biology

I - Core Principles

Year Set 3

	Question Number							
Specification Section	1	2	3	4	5	6	7	Total
10.1 Biological molecules	8	3						11
10.2 Cells			7	8				15
10.3 Cell transport					8			8
10.4 Exchange of materials								
10.5 Enzymes		3						7
10.6 Digestion						14	15	29

Total 66

	Question Number							
Assessment Objective	1	2	3	4	5	6	7	Total
AO1								
Knowledge with Understanding	5	6	6	5	6	7	7	42
AO2								
Application/Analysis/Evaluation	3		1	3	2	7	8	24

Total 66

Mark Schemes for Question Paper

Biology

I - Core Principles

Year Set 3

Instructions

; = 1 mark / = alternative response

Section A

Question 1

A solution of unknown composition is tested with Benedict's solution and a positive result is obtained.

- a) Describe a positive result with the Benedict's test.

green / yellow/ red;

precipitate;

(2)

- b) Explain why it would be wrong to deduce that the positive Benedict's test indicates that glucose is present.

Benedict's test is a test for reducing sugars;

there are reducing sugars other than glucose;

e.g. fructose, maltose, and lactose;

3)

- c) Explain why it would not be correct to deduce from a positive Benedict's test that there were no disaccharides present in the solution.

sucrose is a non-reducing sugar;

which gives a negative result with the Benedict's test;

they could be present 'hidden' by the positive result for reducing sugars;

(3)

Total 8

Question 2

- a) Give the names of two proteins in the body.
any two proteins, e.g. keratin, amylase, collagen, haemoglobin etc; (1)
- b) Describe the biuret test for proteins, and the appearance of a positive result.
add an equal volume of (5%) potassium hydroxide;
add 2 drops of 1% copper sulphate;
mauve or purple colour = positive; (2)
- c) Explain how the shape of the three dimensional tertiary structure of an enzyme is essential for its role as a biological catalyst.
the three dimensional tertiary structure is unique to a protein;
determines the shape of the 'active site';
specific active site needed to combine with a specific substrate; (3)

Total 6

Question 3

- a) Plant and animal cells are both described as eukaryotic cells, describe the main features of eukaryotic cells.
- presence of a membrane bound nucleus at some stage in the cell cycle;
 - with chromosomes;
 - mitochondria;
 - chloroplastids in plant cells;
 - endoplasmic reticulum;
 - Golgi apparatus;
 - cellulose cell wall in plants;
- (4)
- b) Name a type of organism which has a prokaryotic cell. State two differences between this prokaryotic cell and a eukaryotic animal cell.
- bacterium;
 - no membrane bound nucleus at any stage in the cell cycle;
 - no chromosomes, strand of DNA only;
 - plasmids;
 - mesosomes;
 - cell wall;

(3)

Total 7

Question 4

- a) In the table below list three differences between the light (optical) and electron microscopes

Table with two three-box columns.

Light (optical) microscope

Electron microscope

Uses light

Uses a stream of electrons

Light focussed by lenses

Electrons focussed by magnets

Objects observed directly

Objects observed via fluorescent screen

(3)

- b) Describe the main difference between the mode of functioning of the transmission and scanning electron microscopes.

Transmission electron microscope - electrons pass through thin sections of specimen

Scanning electron microscope - electrons reflected from surface of specimen

(2)

- c) Drawing X represents cilia. Drawing Y represents the appearance of a section of cilia.

Interpret and explain the difference in appearance between the two cell diagrams.

cilia elongated cylindrical relatively long structures;

thin sections along their length only cut small lengths;

and rarely 'catch' the connection to the cell;

giving appearance of unattached oval segments;

(3)

Total 8

Question 5

- a) i) What is the water potential of pure water under normal conditions?

0 (nought / zero)

(1)

- ii) Explain why alternatives to the term the 'concentration of water' have been devised.

water is the solvent in which solutes dissolve;

the term concentration normally refers to the solute;

therefore the 'term concentration of water' implies it is acting as a solute;

(2)

- iii) Name a factor to which the rate of osmosis is proportional.

temperature / surface area over which it is occurring / concentration gradient /
water potential gradient;

(1)

- b) If a plant cell is placed in pure water, it eventually becomes fully turgid. The cellulose cell wall can expand no further and so no more water enters the cell. Explain this process in terms of the effect of pressure on water potential.

the pressure inside the cell is raised above that in a non-turgid cell;

an increase in pressure increases the water potential;

reducing the water potential gradient to zero;

so no more water enters the cell

(4)

Total 8

Section B

Answer all the questions in the spaces provided.

Answers should be written in continuous prose wherever appropriate.

Quality of written communication will be assessed in these answers.

Question 6

The relationship between the size of an organism or structure and the surface area to volume ratio is significant in the exchange of substances and heat. The nomogram below enables the surface area of an individual to be estimated from their height and weight.

- a) Estimate the surface area of the two individuals X and Y:

i) X - 2.0 m^2 ; (1)

ii) Y - 1.74 m^2 ; (1)

- b) Making an oversimplified assumption that body mass is directly proportional to body volume, use the figures to describe which of the two individuals would have the higher heat production all other physiological factors being equal) when both were exposed to the same environmental conditions including an external temperature of 10°C with no special protective clothing.

with an external temperature of 10°C ;

heat lost by the body (37°C);

X with the greater surface area will lose more heat than Y;

heat generated by the metabolism of the volume of the body;

therefore surface area to volume ratio important;

X has largest $\text{SA}/\text{V} = 2.0/71 = 0.028$;

Y $\text{SA}/\text{V} = 1.74/72 = 0.024$;

therefore X has smaller SA/V and will have the higher heat production rate;

as the body compensates for the heat loss; (8)

- c) The surface area to volume ratio is particularly important for organisms which exchange gases over their skin surface. Briefly describe the similarities with regard to the uptake of oxygen in these organisms with the example of heat exchange dealt with in part (b).

both exchanges over the surface area;

to and from the volume of the body;

therefore SA/V important; (3)

- d) Adult common frogs exchange gases over their skin surface, but also have lungs. With reference to the fact that there is much less oxygen in a given volume of water than in the same volume of air, explain when the lungs of a frog are most important for oxygen uptake.

lungs most important when frog is in water;

supplements its surface uptake of oxygen;

by breathing air at the surface; (2)

Total 15

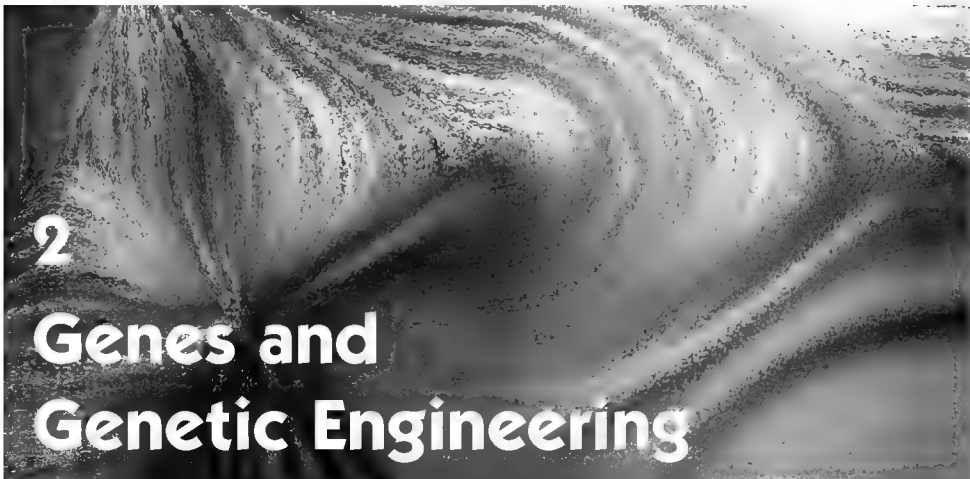
Question 7

There are many different chromatography techniques, but the original one is paper chromatography. The initial set up for a simple separation using paper chromatography is shown below.

- a) Calculate the R_f value for substance X. Show your workings.
Distance moved by X (leading edge) = 54 mm
Distance moved by solvent front = 59 mm
 $R_f = 54/59 = 0.92$ (4)
- b) Describe what extra treatment of the paper chromatogram would be necessary if the substance under investigation was not a pigment.
treatment to locate the invisible separated substances;
e.g. ninhydrin spray for amino acids;
radioactive labels and X ray film; (2)
- c) In two way chromatography a chromatogram which has undergone one way chromatography described previously, is turned on its side and the process repeated using a different solvent as shown below.
- i) Describe the purpose of two way chromatography, and explain why a solvent different from that used in the first run must be used in the second run.
to separate out substances that had similar R_f values in the first solvent;
if same solvent used in second run same problem of non-separation remains; (2)
- ii) In addition to the use of R_f values, substances on a chromatogram may be identified by comparison with the positions of known substances as shown below.
In the example shown on the diagrams above, identify substance q.
q = substance Y; (1)
- d) Explain the principles upon which paper chromatography is based.
separation / partition / relative solubility in;
solvent phase and aqueous phase (water) held on cellulose of paper;
substances more soluble in the solvent than in water travel the furthest;
by capillary action of the solvent in the paper;
characteristic of particular substances; (4)
- e) Explain why touching the chromatography paper before starting a run to investigate a sample of amino acids would invalidate (undermine) the results.
skin / cells / sweat contain amino acids;
contaminate sample under investigation; (2)

Total 15

Exam style question papers



2.1 The Genetic Code

2.2 The Cell Cycle

2.3 Sexual Reproduction

2.4 Application of Gene Technology

Examination Style Question Papers

Name: _____

Biology

2 - Genes and Genetic Engineering

Year Set 3

Time allowed | hour 15 minutes

Instructions:

- Answer all the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Question 1

Enzymes are proteins whose synthesis is controlled by DNA. Some control metabolic pathways within the body cells and therefore influence the phenotype of an organism. A simple diagram of a metabolic pathway is shown below



a) Distinguish between:

i) intracellular enzymes and extracellular enzymes;

(2)

ii) describe the relationship between the DNA content (genotype) of an organism and its phenotype;

(3)

b) Describe the phenotype which will result, if the enzyme controlling step 2 does not function.

(1)

Total 6

Question 2

A length of DNA which codes for a protein is known as a gene. Explain the following observations in terms of protein enzymes controlling metabolic pathways.

- a) Genes can be dominant, ie only one copy per diploid set of chromosomes in each cell is required to exert its effect.

(2)

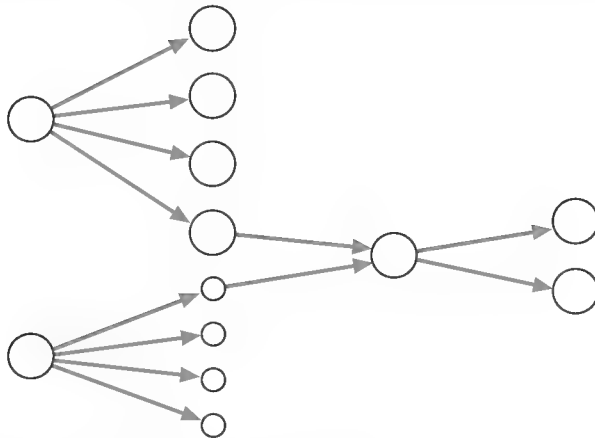
- b) Genes can be recessive, ie where the effect is only exerted if two copies are present.

(2)

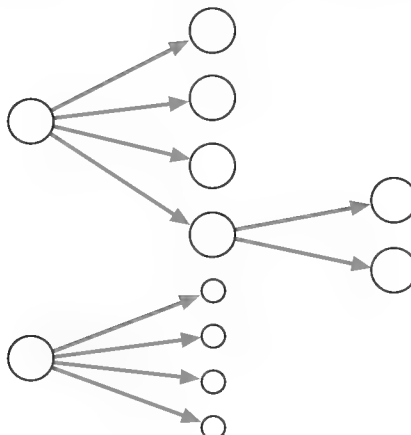
Total 4

Question 3

Study the simplified diagram below of the life cycle of a mammal which routinely produces twin offspring.



- a) Label the diagram to show where the following events occur:
- i) mitosis (1)
 - ii) meiosis (1)
 - iii) fertilisation (1)
- b) On the diagram above, indicate the chromosome number as being either $2N$ (diploid) or N (haploid) in the circles representing nuclei of typical cells at particular stages. (2)
- c) The diagram below, represents a life cycle, common in some organisms, in which development of eggs into offspring can occur without fertilisation. Indicate the chromosome number as being either $2N$ (diploid) or N (haploid) in the circles representing nuclei of typical cells at particular stages.



(2)

Total 7

Question 4

High energy radiation and some chemicals are mutagenic agents; that is they increase the rate of gene mutation. Some figures are listed below for the major sources of radiation to which humans are exposed are listed below.

Sources and average worldwide doses of radiation (millisieverts)	
Cosmic rays	0.39 (roughly doubling with every 2000 feet of altitude)
diet	0.23
medical	0.30
gamma rays (ground & building)	0.46
radon gas	1.30 (highest in houses built on and with granite limestone)

- a) Some believe that there is a dose threshold below which there is no measurable risk, others believe in a linear or no-threshold risk, ie that there is no safe lower limit.

- i) Give an explanation which would support the idea of a dose threshold.

(2)

- ii) Give an explanation which would support the idea of a linear threshold risk.

(2)

- b) Radiation can affect DNA directly by breaking the strands, and by ionising water which gives highly reactive hydroxide ions OH⁻ which can change the bases. Explain how breaking the strands of DNA could lead to mutations.

(3)

Total 7

Question 5

When only traces of DNA are available for analysis, for example in forensic science, DNA amplification is necessary to make sufficient quantities of DNA identical to the trace samples. This is achieved by the polymerase chain reaction or PCR, which can give rise to billions of copies of a DNA sample in a few hours as a result of exponential replication or 'compound interest'.

- a) i) Briefly explain the principle of exponential replication or 'compound interest' by which such rapid amplification can be achieved.

(2)

- ii) Describe when this process occurs in normal cell division.

(2)

The details of the polymerase chain reaction include the following steps.

Trace DNA strands are separated by heating at 98°C for 5 minutes.

Nucleotides (A, T, C, G,), DNA polymerase, and 'Primers' of short strands of RNA are added.

When cooled to 60°C the primers attach to the single strands of DNA and provide a starting sequence, and DNA polymerase catalyses replication of complementary strands of the DNA.

- b) i) Identify the enzyme in the process and explain the derivation of its name.

(1)

- ii) Explain the role of the nucleotides in the process.

(1)

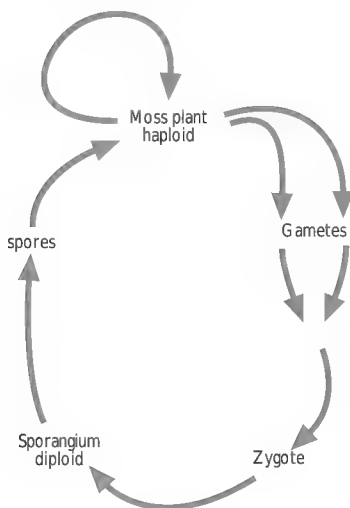
- c) Describe precisely from where the traces of DNA would have come in a drop of dried blood.

(1)

Total 7

Question 6

Study the diagram below of the life cycle of a moss plant and answer the following questions.



a) Mark on the diagram where the following processes occur.

i) meiosis (1)

ii) fertilisation (1)

b) The Fern life cycle is very similar but the fern plant is diploid, and produces spores on the undersurface of its leaves.

i) Describe where meiosis occurs in the fern.

(1)

c) All plants have life cycles based on the pattern seen in mosses and ferns. With regards to meiosis and fertilisation explain how the life cycles of multicellular animals differ from those in plants.

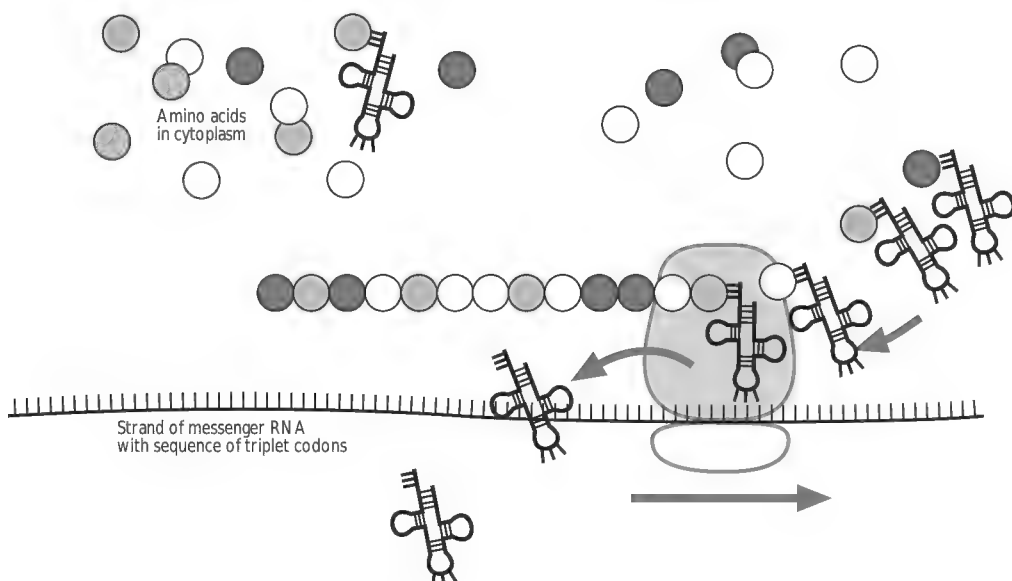
(2)

Total 5

Section B

Question 7

Study the diagram below of protein synthesis and answer the following questions.



a) On the diagram above label the following:

- i) a transfer RNA (tRNA) molecule; (1)
- ii) an anti-codon; (1)
- ii) the ribosome; (1)
- iii) the polypeptide; (1)

b) With regard to the formation of a polypeptide chain:

- i) define the term 'translation';

(2)

- ii) What is the function of the ribosome (rDNA);

(1)

Question continued...

iv) Which nucleotide base is present in RNA but not DNA?

(1)

v) describe how a polypeptide chain gives rise to an active protein enzyme molecule.

(3)

c) Explain the significance in protein synthesis of the fact that the genetic code is 'non-overlapping'.

(4)

Total 15

Question 8

Study the information regarding cystic fibrosis set out below and answer the following questions.

Cystic fibrosis is a serious condition which results from the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane. Symptoms of the condition include thick mucus which blocks airways and the pancreatic duct.

1 in 20 caucasians are carriers

1 in 2500 are born with the disease

the gene for cystic fibrosis is on chromosome 7 of the 23 in a haploid human set

the relevant protein is the Cystic Fibrosis Transmembrane Regulator

the protein consists of 1480 amino acids

the mutation is a deletion of 3 nucleotides

so that the protein lacks phenylalanine at position 508

Techniques that might be used to introduce healthy CFTR genes into lung epithelial cells include; the use of harmless virus into which a gene has been inserted, or the wrapping of the gene in lipid molecules so that it can enter the lung cells more easily.

- a) i) Explain why there are more carriers than sufferers of cystic fibrosis

(1)

- ii) How many chromosomes carrying the gene will there be in each body cell? Explain your answer.

(2)

- b) Explain how the lack of a correctly functioning transmembrane protein can arise from a deletion of three nucleotides in the DNA.

(4)

Question continued...

-
- c) Explain how the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane can cause the symptom of thick mucus.

(4)

- d) Explain why wrapping the engineered gene in lipid molecules helps it pass into the lung cells more easily

(4)

Total 15

Assessment Grid

Biology

2 - Genes and Genetic Engineering

Year Set 3

	Question Number								
Specification Section	1	2	3	4	5	6	7	8	Total
11.1 Genetic code	6						15		21
11.2 Cell cycle		4							4
11.3 Sexual reproduction			7			5			12
11.4 Gene Technology				7	7			15	29
Total 66									

	Question Number								
Assessment Objective	1	2	3	4	5	6	7	8	Total
AO1									
Knowledge with Understanding	6		5	5	4	2	11	7	40
AO2									
Application/Analysis/Evaluation		4	2	2	3	3	4	8	26
Total 66									

Mark Schemes for Question Paper

Biology

2 - Genes and Genetic Engineering Year Set 3

Instructions

; = 1 mark / = alternative response

Section A

Question 1

Enzymes are proteins whose synthesis is controlled by DNA. Some control metabolic pathways within the body cells and therefore influence the make up (phenotype) of an organism. A simple diagram of a metabolic pathway is shown below

- a) Distinguish between:
- i) intracellular enzymes and extracellular enzymes;
intracellular enzymes remain inside cells control metabolic pathways);
extracellular enzymes secreted from cells (e.g. digestive enzymes); (2)
 - ii) describe the relationship between the DNA content (genotype) of an organism and its phenotype;
expression of the genotype influenced by the environment;
in which the genotype is expressed;
during development of the organism;
phenotype = genotype + environment; (3)
- b) Describe the phenotype which will result if the enzyme controlling step 2 does not function.
no pigment / albino; (1)

Total 6

Question 2

A length of DNA which codes for a protein is known as a gene. Explain the following observations in terms of protein enzymes controlling metabolic pathways.

- a) Genes can be dominant ie only one copy per diploid set of chromosomes in each cell is required to exert its effect.
one copy of the active gene is sufficient to produce the enzyme;
therefore metabolic pathway functions; (2)
- b) Genes can be recessive where the effect is only exerted if two copies are present.
enzyme must be absent before pathway is interrupted
two non-functional versions of the gene result in no enzyme (recessive); (2)

Total 4

Question 3

Study the simplified diagram of the life cycle of a mammal which routinely produces twin offspring.

- a) Label the diagram to show where the following events occur.
- i) mitosis (1)
 - ii) meiosis (1)
 - iii) fertilisation (1)
- b) On the diagram above, indicate the chromosome number as being either 2N (diploid) or N (haploid) in the circles representing nuclei of typical cells at particular stages (2)
- c) The diagram below, represents a life cycle, common in some organisms, in which development of eggs into offspring can occur without fertilisation. Indicate the chromosome number as being either 2N (diploid) or N (haploid) in the circles representing nuclei of typical cells at particular stages. (2)

Total 7

Question 4

High energy radiation and some chemicals are mutagenic agents, that is they increase the rate of gene mutation. Some figures are noted below for the major sources of radiation to which humans are exposed are listed below.

- a) i) Some believe that there is a dose threshold below which there is no measurable risk, others believe in a linear or no-threshold risk, ie that there is no safe lower limit. Give an explanation which would support the idea of a dose threshold.

doses below threshold are dispersed harmlessly on path through body;

DNA repair mechanisms can keep up with level of damage up to threshold;

(2)

- ii) Give an explanation which would support the idea of a linear threshold risk.

all radiation is damaging;

can be no 'threshold' below which all damage suddenly ceases;

(2)

- b) Radiation can affect DNA directly by breaking the strands, and by ionising water which gives highly reactive hydroxide ions $\text{OH}^\cdot$ which can change the bases. Explain how breaking the strands of DNA could lead to mutations.

genes are precise sequences of bases over a length of DNA;

if DNA broken within a gene the gene is damaged ie a mutation;

by deletion or inversion if segment rejoins the wrong way round;

(3)

Total 7

Question 5

When only traces of DNA are available for analysis, for example in forensic science, DNA amplification is necessary to make sufficient quantities of DNA identical to the trace samples. This is achieved by the polymerase chain reaction or PCR, which can give rise to billions of copies of a DNA sample in a few hours as a result of exponential replication or 'compound interest'.

- a) i) Briefly explain the principle of exponential replication or 'compound interest' by which such rapid amplification can be achieved.

DNA molecule double every division;

e.g. $2 > 4 > 8 > 16 > \text{etc};$

(2)

- ii) Describe when this process occurs in normal cell division.

mitosis;

interphase in replication of DNA;

(2)

The details of the polymerase chain reaction include the following steps.

Trace DNA strands are separated by heating at 98°C for 5 minutes.

Nucleotides (A, T, C, G), DNA polymerase, and 'Primers' of short strands of RNA are added.

When cooled to 60°C the primers attach to the single strands of DNA and provide a starting sequence, and DNA polymerase catalyses replication of complementary strands of the DNA.

- b) i) Identify the enzyme in the process and explain the derivation of its name.

DNA polymerase the replication of DNA is achieved by polymerisation;

(1)

- ii) Explain the role of the nucleotides in the process.

they are the building blocks (monomers);

(1)

- c) Describe precisely from where the traces of DNA would have come in a drop of dried blood.

nuclei of white cells;

(1)

Total 7

Question 6

Study the diagram below of the life cycle of a moss plant and answer the following questions.

- a) Mark on the diagram where the following processes occur.
- i) meiosis
before spore formation; (1)
 - ii) fertilisation
before zygote formation; (1)
- b) The Fern life cycle is very similar but the fern plant is diploid, and produces spores on the undersurface of its leaves.
- i) Describe where meiosis occurs in the fern.
in spore mother cells on the undersurface of its leaves; (1)
- c) All plants have life cycles based on the pattern seen in mosses and ferns. With regards to meiosis and fertilisation explain how the life cycles of multicellular animals differ from those in plants.
- in plants meiosis precedes spore formation;
 - in animals meiosis precedes gamete formation; (2)

Total 5

SECTION B

Question 7

Study the diagram below of protein synthesis and answer the following questions.

- a) On the diagram above label the following:
- i) a transfer RNA (tRNA) molecule; (1)
 - ii) an anti-codon; (1)
 - iii) the ribosome; (1)
 - iv) the polypeptide; (1)
- b) With regard to the formation of a polypeptide chain:
- i) define the term 'translation';
the conversion of the genetic code on the mRNA at the ribosomes;
into the reality of a polypeptide chain in the cytoplasm; (2)
 - ii) What is the function of the ribosome (rRNA)?
It organises synthesis of the peptide chain; (1)
 - iii) Which nucleotide base is present in RNA but not DNA?
Uracil; (1)
 - iv) describe how a polypeptide chain gives rise to an active protein enzyme molecule.
as it gets longer, attractions between parts of the chain;
e.g. forming hydrogen bonds;
result in folding of chain;
complex three dimensional shape formed with an active site; (3)
- c) Explain the significance in protein synthesis of the fact that the genetic code is 'non-overlapping'.
the genetic code can only be read from a specific starting point;
which fixes the base sequences within each triplet codon which is 'read' by mRNA;
therefore each strand of mRNA produces one specific polypeptide strand;
if there was no fixed 'start' codon, subsequent codons would be 'read' differently;
the bases on one codon cannot 'overlap' into another; (4)

Total 15

Question 8

Study the information on cystic fibrosis set out below and answer the following questions.

Cystic fibrosis is a serious condition caused as a result of the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane. Symptoms include thick mucus which blocks airways and the pancreatic duct.

1 in 20 caucasians are carriers

1 in 2500 are born with the disease

the gene for cystic fibrosis is on chromosome 7 of the 23 in a haploid human set the relevant protein is the Cystic Fibrosis Transmembrane Regulator

the protein consists of 1480 amino acids

the mutation is a deletion of 3 nucleotides

so that the protein lacks phenylalanine at position 508

Techniques that might be used to introduce healthy CFTR genes into lung epithelial cells include; the use of harmless virus into which a gene has been inserted or the wrapping of the gene in lipid molecules so that it can enter the lung cells more easily.

- a) i) Explain why there are more carriers than sufferers of cystic fibrosis
cystic fibrosis gene is recessive; (1)
- ii) How many chromosomes carrying the gene will there be in each body cell? Explain your answer.
two;
chromosomes occur in pairs in body cells; (2)
- b) Explain how the lack of a correctly functioning transmembrane protein can arise from a deletion of three nucleotides in the DNA.
triplet codon codes for an amino acid;
deletion of codon results in an amino acid missing from the sequence in a polypeptide /protein;
missing amino acid results in change of structure of protein;
in this case the transmembrane protein which controls the transport of chloride ions; (4)
- c) Explain how the lack of a correctly functioning transmembrane protein which controls the transport of chloride ions across the cell membrane can cause the symptom of thick mucus.
active transport of chloride ions out of the cells;
followed by passive movement of water by osmosis;
lack of transport of chloride ions reduces water movement out of cells;
'dry' membranes / thick mucus produced by glandular cells; (4)
- d) Explain why wrapping the engineered gene in lipid molecules helps its passage into the lung cells.
enters cell by cell surface membrane;
which is lipid (fatty) in nature;
lipids mix freely with each other;
speeding passage across membrane into cell; (4)

Total 15

Exam style question papers



3.1 Transport Systems

3.2 The Control of Breathing and Heartbeat

3.3 Energy and Exercise

3.4 The Transport of Substances in Plants

Examination Style Question Papers

Name: _____

Biology

3 - Physiology & Transport

Year Set 3

Time allowed | hour 15 minutes

Instructions:

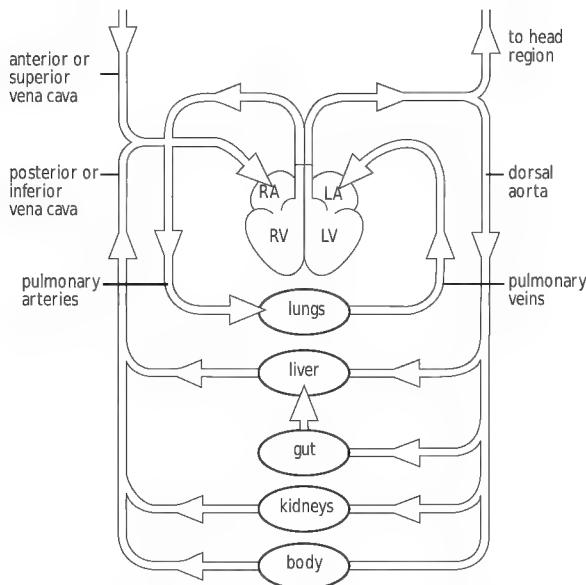
- Answer all the questions in the spaces provided.

Information

- Mark allocations are shown in brackets.
- The maximum mark for this paper is 66.
- About 40 of the marks will be for knowledge and understanding; the remainder of will be for application of knowledge and understanding, analysis and evaluation
- Answers for Section A are expected to be short and precise.
- Section B questions should be answered in continuous prose where appropriate. Quality of Written Communication will be assessed in answers to these questions. Scientific terminology should be used where appropriate.
- A calculator may be used.

Question 1

Study the diagram of the general pattern of blood circulation in a mammal, and answer the following questions.



a) Label the diagram to identify by name the blood vessels entering the liver and kidneys. (2)

b) i) Describe the pathway of the blood from the right ventricle to the kidneys.

(2)

ii) Explain why the liver receives blood in two different major blood vessels.

(2)

c) Identify on the diagram with the letters P, Q, and R those vessels in which the blood:

i) P - has the highest urea content;

(1)

ii) Q - has the lowest urea content;

(1)

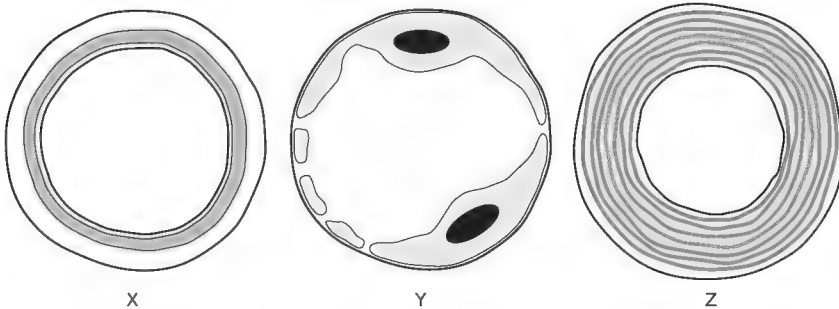
iii) R - has a variable glucose content;

(1)

Total 9

Question 2

The three diagrams below are of cross (transverse) sections of an artery, vein and capillary, not drawn to scale.



a)

i) Identify the artery, vein and the capillary.

(1)

ii) What is the diameter of an average capillary?

(1)

b)

Describe how the structure of the capillary is adapted to its function.

(3)

c)

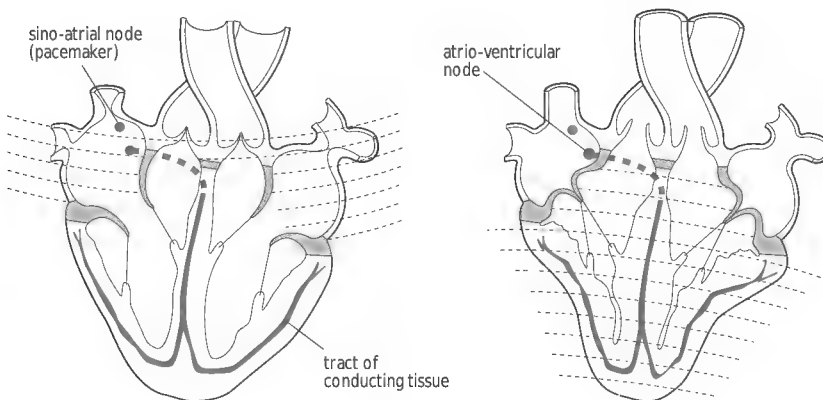
Describe a difference in the structure of arteries and veins which is usually only seen in sections along the length of the vessel (longitudinal sections), and explain why this difference is rarely seen in cross sections

(3)

Total 8

Question 3

The diagrams below show vertical sections of the heart at different stages of the cardiac cycle. The spread of electrical activity through the walls is indicated by the radiating curved lines.



- a) Draw arrows on the diagram above to show the directions in which electrical activity spreads through the walls of the heart. (1)

- b) Relate this pattern of spread of electrical activity, and the resultant contractions of the atria and ventricles in the cardiac cycle, to the direction of the blood flow through the heart.

(3)

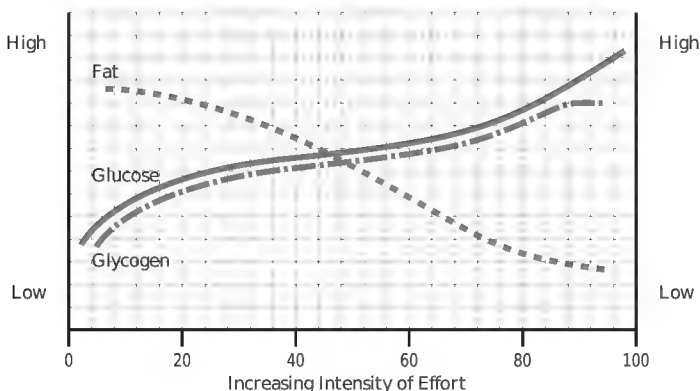
- c) Explain what causes the heart valves to open and close during the cardiac cycle.

(2)

Total 6

Question 4

The graph below shows the relationship between the use of glucose, glycogen and triglycerides (fat) as sources of energy for muscle contraction, with increasing intensity of effort.



- a) Explain why the curves for glucose and glycogen follow each other so closely.

(3)

- b) Explain why the use of triglycerides (fat) decreases with increasing intensity of effort.

(2)

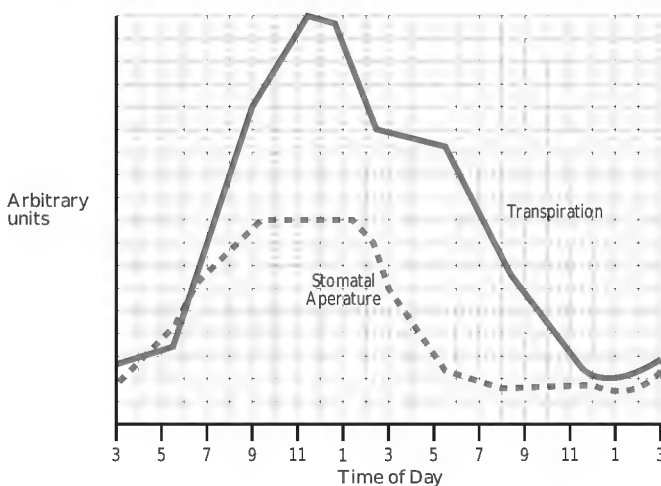
- c) Describe a type of exercise which would maximise the use of triglycerides (fat) as the source of energy for that exercise.

(2)

Total 7

Question 5

The graph below show the relationship between the degree of opening of the stomata (stomatal aperture) and the rate of transpiration over a 24 hour period, under natural conditions on a summer's day.



- a) i) Describe the general relationship between the stomatal aperture and the rate of transpiration.

(2)

- ii) Explain how the transpiration rate can rise and fall independent of the stomatal aperture.

(3)

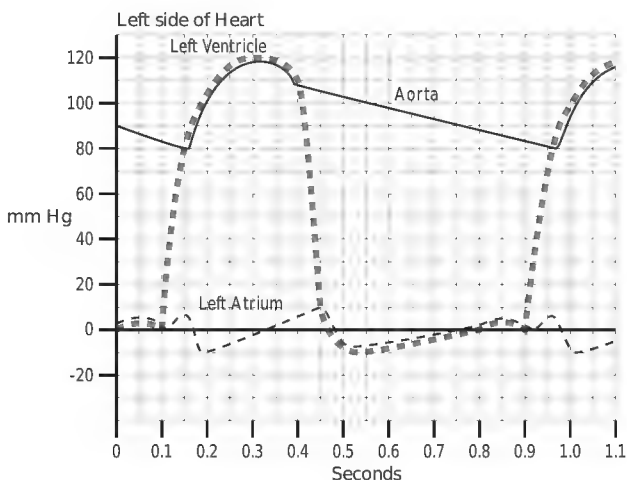
- b) Some plants e.g. desert plants, close their stomata during the day and open them at night. Explain the advantage of this pattern of opening and closing to these plants.

(2)

Total 7

Question 6

Study the diagram showing the pressure changes in the left side of the heart and the main aorta and answer the following questions.



- a) i) Mark on the horizontal time axis the period during which the left ventricle is contracting. (1)

ii) Describe the state of the bicuspid and pocket valves in this period.

 _____ (2)

- b) Describe how the cycle of pressure changes in the left ventricle is related to the pulse.

 _____ (3)

- c) i) From the graph deduce the highest (systolic) value, and the lowest diastolic) value, for the blood pressure measured in the arteries of the left arm.

 _____ (2)

ii) Calculate the heart rate of the individual under test, and explain your reasoning.

 _____ (2)

Question continued...

- d) Describe and explain the changes in the graph that would be seen during progressive aerobic exercise on a treadmill.

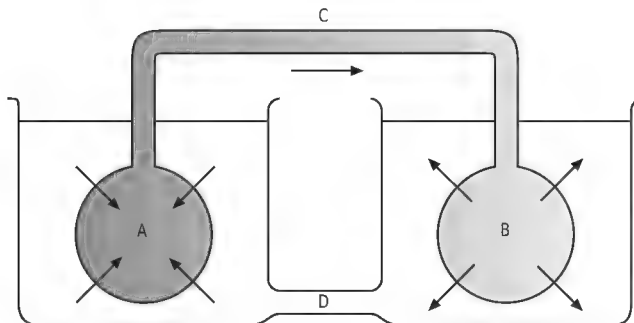
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(6)

Total 16

Question 7

The diagram below is of a simple model used to demonstrate the mass flow hypothesis for the translocation of sugars in the phloem.



a) Identify which part of the model represents:

i) the phloem;

(1)

ii) the xylem;

(1)

b) For the movement of water and dissolved sugars to be maintained from chamber A to chamber B, sugars must be continually added to A and removed from B. Identify the regions equivalent to A and B and the processes that achieve these changes in the plant.

(2)

c) i) Ringing experiments remove all tissues external to the xylem in a woody stem and lead to the accumulation of sugars above the ring. Explain how this helps to demonstrate that translocation of sugars occurs in the phloem.

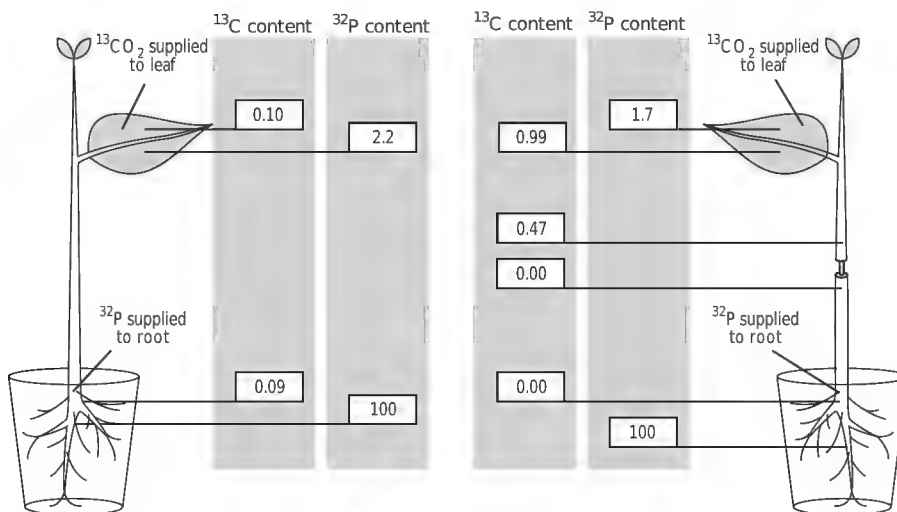
(2)

ii) Metabolic inhibitors such as, temperatures above and below the optimum, respiratory poisons such as cyanide, and anaerobic conditions all result in a slowing of the rate of translocation. Explain how this evidence supports the view that translocation occurs in the phloem.

(2)

Question continued...

- d) The diagrams below represent the results of ringing experiments on plants. The leaves of which were exposed to radioactive $^{13}\text{CO}_2$ and the roots of which were exposed to radioactive ^{32}P .



- i) By reference to the figures explain how this experiment demonstrates the role of phloem in the translocation of sugars, and that of the xylem in the transport of inorganic ions from the roots.

(3)

- ii) Studies with radioactive tracers have indicated rates of flow of sugars in the phloem of 1 metre per hour in grasses, and as high as 50 metres per hour in soya plants. Explain how this raises question marks about the mass flow hypothesis of translocation of sugars in the phloem.

(2)

Total 13

Assessment Grid

Biology

3 - Physiology & Transport

Year Set 3

	Question Number							
Specification Section	1	2	3	4	5	6	7	Total
12.1 Transport systems	9	8				16		33
12.2 Breathing and heartbeat			6					6
12.3 Energy and exercise				7				7
12.4 Transport in plants					7		13	20

Total 66

	Question Number							
Assessment Objective	1	2	3	4	5	6	7	Total
AO1								
Knowledge with Understanding	4	6	5	4	4	10	8	41
AO2								
Application/Analysis/Evaluation	5	2	1	3	3	6	5	22

Total 66

Mark Schemes for Question Paper

Biology

3 - Physiology & Transport Year Set 3

Instructions

; = 1 mark / = alternative response

Question 1

Study the diagram of the general pattern of blood circulation in a mammal, and answer the following questions.

- a) Label the diagram to identify the blood vessels entering the liver and kidneys.
hepatic artery hepatic portal vein
renal artery; (2)
- b) i) Describe the pathway of the blood from the right ventricle to the kidneys.
to lungs;
to left atrium;
to left ventricle;
to main aorta;
to renal arteries to the kidneys; (2)
- ii) Explain why the liver receives blood in two different major blood vessels.
in the hepatic artery for its own supplies of oxygen;
in the hepatic portal vein from the gut carrying nutrients for adjusting the level of; (2)
- c) Identify on the diagram with the letters P, Q, and R those vessels in which the blood:
- i) P - hepatic vein; (1)
- ii) Q - renal vein; (1)
- iii) R - hepatic portal vein; (1)

Total 9

Question 2

The three diagrams below are of cross (transverse) sections of an artery, vein and capillary, not drawn to scale.

- a) i) Identify the artery, vein and the capillary.

X - Vein

Y - Capillary

Z - Artery

(1)

- ii) What is the diameter of an average capillary?

6 - 8 μm

(1)

- b) Describe how the structure of the capillary is adapted to its function.

wall one cell thick;

gaps between cells;

some are fenestrated with pores;

all features enable / reduce resistance to formation of tissue fluid;

diameter same as that of the red blood cells;

ensures close proximity of red blood cells and tissues;

(3)

- c) Describe a difference in the structure of arteries and veins which is usually only seen along the length of the vessel (longitudinal sections) and explain why this difference is rarely seen in cross sections.

veins have pocket valves;

only at intervals along their length;

therefore greater chance of a cross section missing them;

(3)

Total 8

Question 3

The diagrams below show vertical sections of the heart at different stages of the cardiac cycle. The spread of electrical activity through the walls is indicated by the radiating curved lines.

- a) Draw arrows on the diagram above to show the directions in which electrical activity spreads through the walls of the heart.

arrows radiating downwards and across in atria and upwards in ventricles (possibly down in septum and up in walls;

(1)

- b) Relate this pattern of spread of electrical activity, and the resultant contractions of the atria and ventricles in the cardiac cycle, to the direction of the blood flow through the heart.

arrow radiating downwards in atria results in downward contraction of atria;

forcing blood downwards into the ventricles;

arrow radiating upwards in ventricles results in upward contraction of ventricles;

forcing blood upwards into pulmonary arteries and main aorta;

(3)

- c) Explain what causes the heart valves to open and close during the cardiac cycle.

heart valves are passive structures incapable of initiating movement;

pressure of blood forces valves open or shut;

dependent upon their one-way arrangement;

(2)

Total 6

Question 4

The graph below shows the relationship between the use of glucose, glycogen and triglycerides (fat) as sources of energy for muscle contraction, with increasing intensity of effort.

- a) Explain why the curves for glucose and glycogen follow each other so closely.
muscle glycogen is the major source;
of glucose for muscle respiration;
as more glucose is oxidised more glycogen is converted; (3)
- b) Explain why the use of triglycerides (fat) decreases with increasing intensity of effort.
triglycerides can only be oxidised in the presence of oxygen;
therefore as oxygen supply to muscles decreases with increasing effort;
less fat can be oxidised; (2)
- c) Describe a type of exercise which would maximise the use of triglycerides (fat) as the source of energy for that exercise.
long duration eg over an hour;
aerobic steady state; (2)

Total 7

Question 5

The graph below show the relationship between the degree of opening of the stomata (stomatal aperture) and the rate of transpiration over a 24 hour period under natural conditions on a summer's day.

- a) i) Describe the general relationship between the stomatal aperture and the rate of transpiration.
as one rises so does the other;
with their highest values around midday;
and their lowest values in the dark; (2)
- ii) Explain how the transpiration rate can rise and fall independent of the stomatal aperture.
once stomata opened maximally no longer limiting factor;
rise and fall of transpiration affected by environmental factors which become limiting;
change in temperature / change in humidity / change in air movements; (3)
- b) Some plants e.g. desert plants, close their stomata during the day and open them at night.
Explain the advantage of this pattern of opening and closing to these plants.
in hot dry conditions danger of excess water loss by transpiration;
via stomata therefore shut during day therefore must open at night for carbon dioxide uptake; (2)

Total 7

Question 6

Study the diagram that shows the pressure changes in the left side of the heart and the main Aorta, and then answer the following questions.

- a) i) Mark on the horizontal time axis the period during which the left ventricle is contracting. (1)
- ii) Describe the state of the bicuspid and pocket valves in this period.
bicuspid valves shut;
pocket valves in the aorta open; (2)
- b) Describe how the cycle of pressure changes in the left ventricle is related to the pulse.
highest pressure in left ventricle;
creates a surge of pressure in the aorta and arteries close to the heart;
pressure drops when left ventricle relaxes;
arteries recoil; (3)
- c) i) From the graph deduce the highest (systolic) value, and the lowest diastolic) value, for the blood pressure measured in the arteries of the left arm.
120 mm Hg;
80 mm Hg; (2)
- ii) Calculate the heart rate of the individual under test, and explain your reasoning.
cardiac cycle from one point to equivalent point in next contraction;
0.8 seconds;
therefore heart rate = $60 / 0.8 = 75$;
beats per minute; (2)
- d) Describe and explain the changes in the graph that would be seen during progressive aerobic exercise on a treadmill.
at the start of the exercise slight increase in pressure peaks;
as exercise progresses time interval between ventricular contractions (systole) shortens;
as heart rate increases;
similarly the width of the pressure curves reduces;
as time for ventricular systole decreases;
complete cycle reduced to as low as 0.2 second;
pressure peaks return to normal / slightly above normal values; (6)

Total 16

Question 7

The diagram below shows a simple model used to demonstrate the mass flow hypothesis for the translocation of sugars in the phloem.

a) Identify which part of the model represents:

i) the phloem;

C;

(1)

ii) the xylem;

D;

(1)

b) For the movement of water and dissolved sugars to be maintained from chamber A to chamber B sugars must be continually added to A and removed from B. Identify the regions equivalent to A and B and the processes that achieve these changes in the plant.

chamber A = leaves carrying out photosynthesis at a rate in excess of respiration in the light;

chamber B = roots carrying out respiration;

(2)

c) i) Ringing experiments remove all tissues external to the cambium in a woody stem and lead to the accumulation of sugars above the ring. Explain how this helps to demonstrate that translocation of sugars occurs in the phloem.

tubular elements (sieve tubes) of phloem found external to cambium in woody twig;

accumulation of sugars synthesised in the leaves above the ring indicate movement down stem interrupted by removal of phloem;

(2)

ii) Metabolic inhibitors such as, temperatures above and below the optimum, respiratory poisons such as cyanide, and anaerobic conditions all result in a slowing of the rate of translocation. Explain how this evidence supports the view that translocation occurs in the phloem.

of the two tubular transport systems in the stem only phloem living;

therefore metabolic inhibitors inhibiting translocation in the phloem;

(2)

d) The diagrams below represent the results of ringing experiments on plants. The leaves of which were exposed to radioactive ^{13}C and the roots of which were exposed to radioactive ^{32}P .

i) By reference to the figures explain how this experiment demonstrates the role of phloem in the translocation of sugars and that of the xylem in the transport of inorganic ions from the roots.

in ringed plant ^{13}C compounds accumulate in the leaf from 0.10 to 0.99;

in ringed plant ^{13}C compounds do not reach roots, unringed 0.09, ringed 0.00;

in ringed plant small effect on transport of ^{32}P to the leaf from 2.2 to 1.7

(3)

ii) Studies with radioactive tracers have indicated rates of flow of sugars in the phloem of 1 metre per hour in grasses, and as high as 50 metres per hour in soya plants. Explain how this could be used as evidence against the mass flow hypothesis of translocation of sugars in the phloem.

only living phloem involved in translocation;

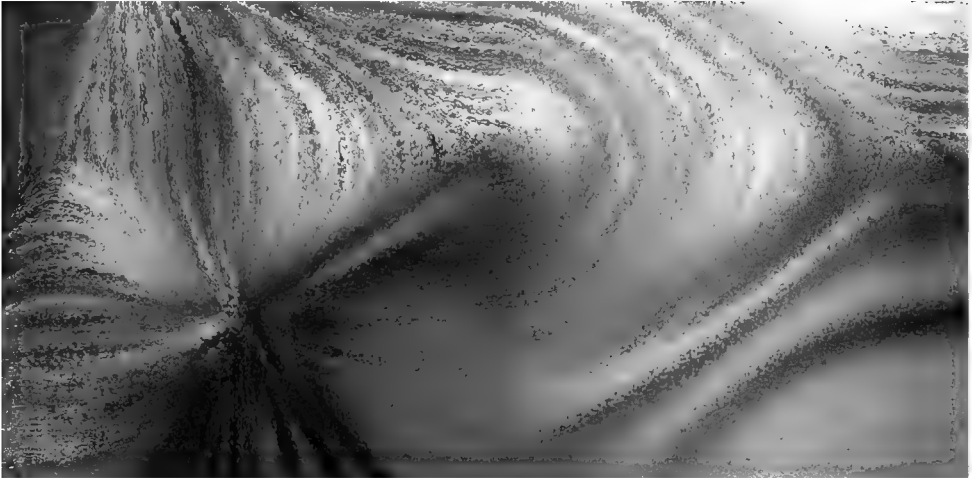
cytoplasm and membranes of sieve tube elements present high resistance to mass flow;

cross section dimensions of sieve tube elements do not seem large enough for mass flow at this rate;

(2)

Total 13

Illustrations



Illustrations

Contents List

1 - Core Principles

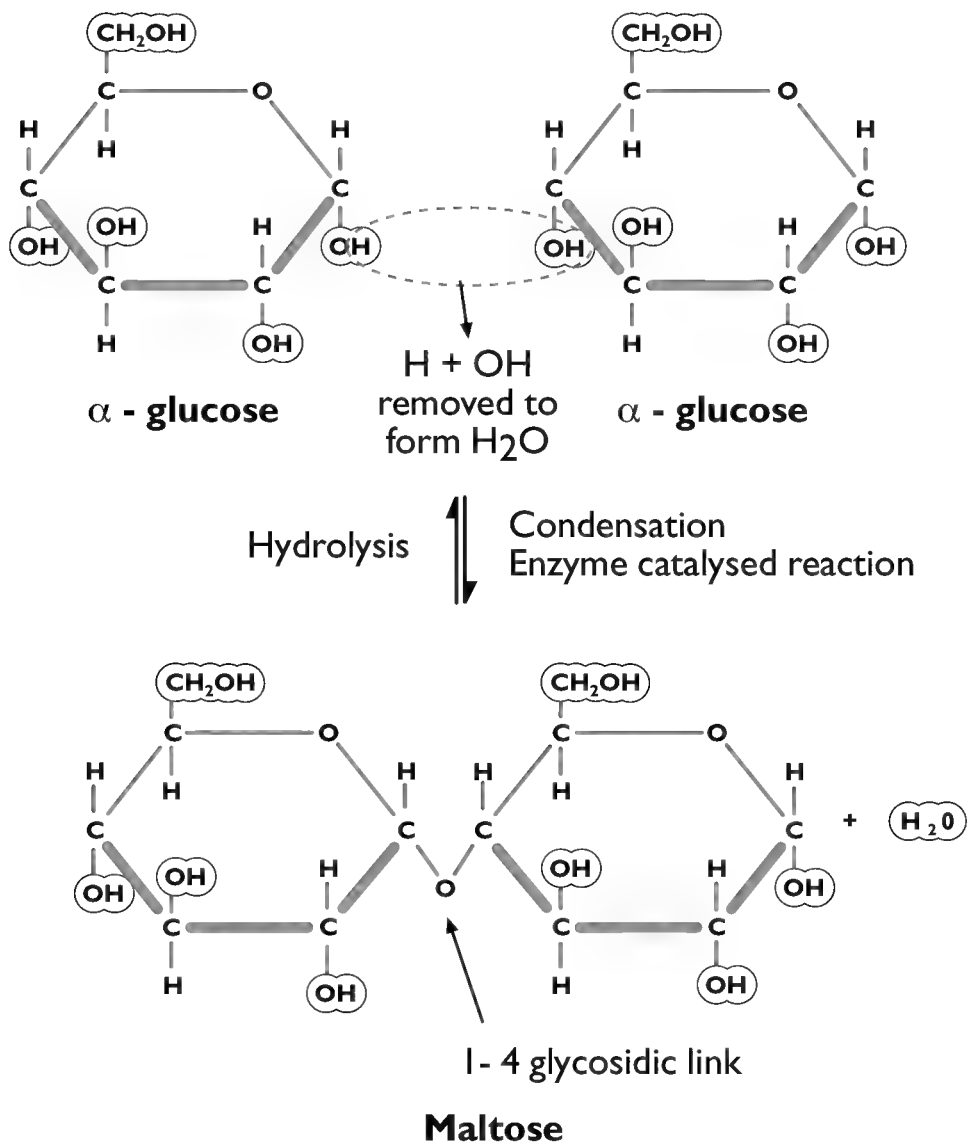
- 1 Monosaccharides and disaccharides
- 2 Polysaccharides
- 3 Amino acid structure and the peptide bond
- 4 Secondary and tertiary structure of proteins
- 5 Structure of haemoglobin
- 6 Structure of triglyceride
- 7 Phospholipids and the bilayer
- 8 Chromatography and chromatograms
- 9 Photomicrograph of animal cells
- 10 Photomicrograph of plant cells
- 11 Animal cell under the Electron Microscope
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- 13 Prokaryotic cell
- 14 Small intestine
- 15 Leaf

2 - Genes & Genetic Engineering

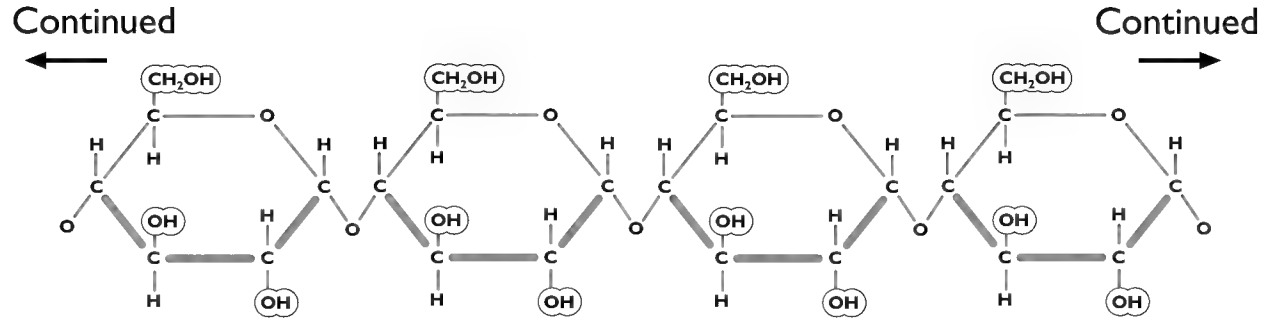
- 16 Phospholipids and the bilayer (as 7)
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- 19 Leaf (as 15)
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- 23 Activation energy
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- 30 Transcription
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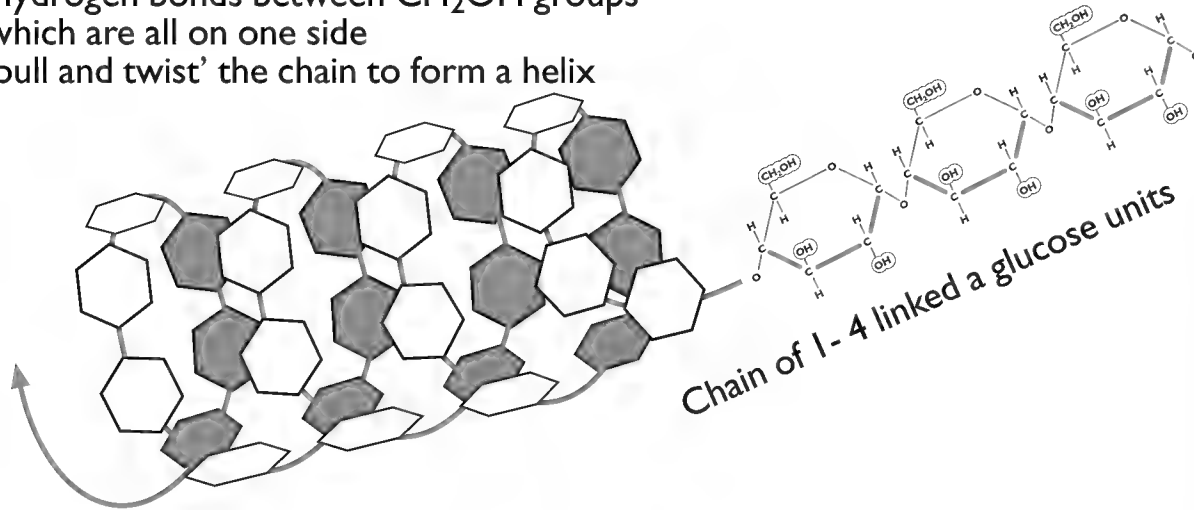
32	Table of amino acid codons
33	Gene mutations
34	Root tip squash
35	Normal human karyotype A
36	Human karyotype analysed into homologous pairs
37	Normal human karyotype B
38	Human karyotype analysed into homologous pairs
39	Meiosis
40	Application of gene technology
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43	Graph/chart of pressure changes during the cardiac cycle
44	Artery and vein
45	Capillaries
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47	Blood and lymph
48	Simple apparatus for the measurement of carbon dioxide in expired air
49	Heart and SA node
50	ATP
51	Ring experiment (not an autoradiograph)

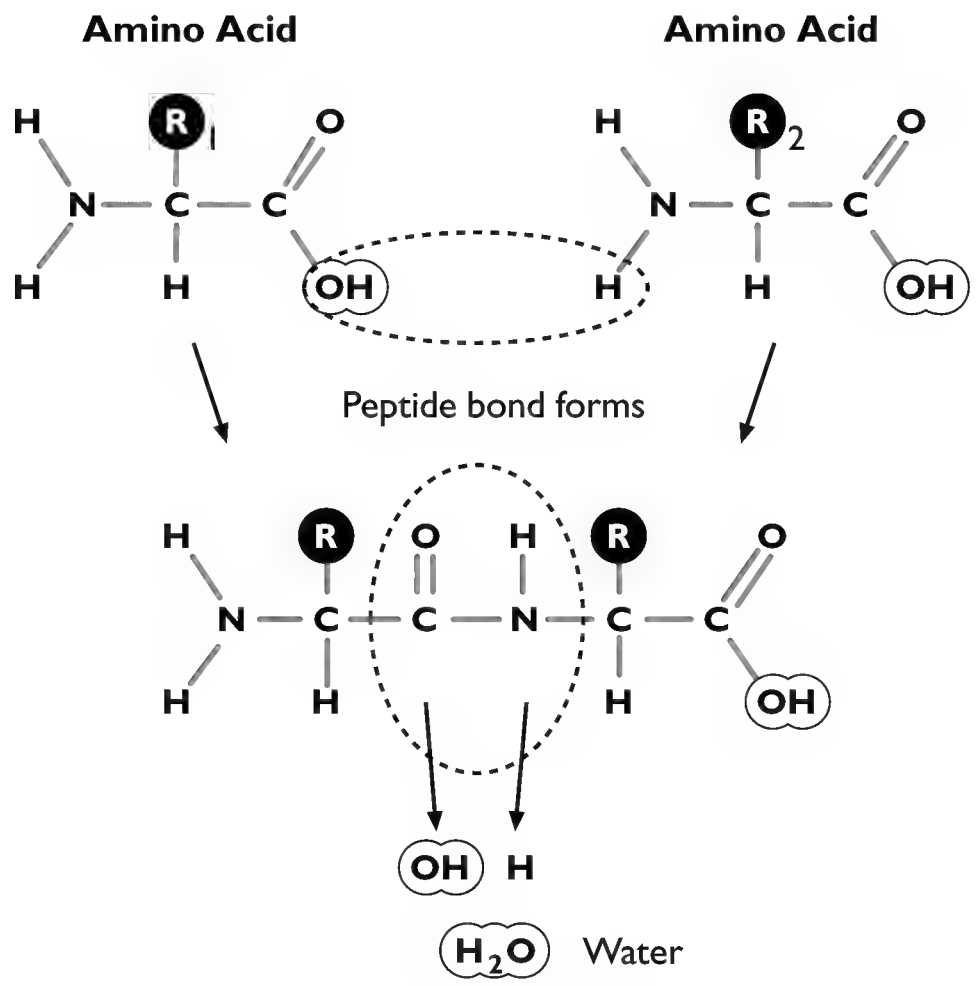


Part of amylose molecule chain



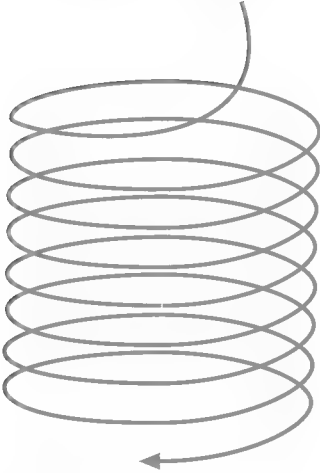
Hydrogen bonds between CH₂OH groups which are all on one side
 'pull and twist' the chain to form a helix



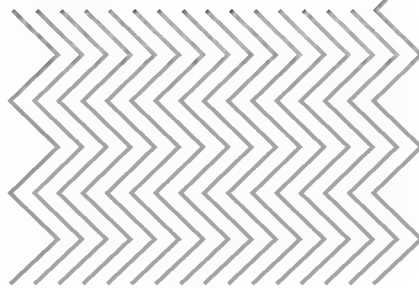


Secondary structure of proteins

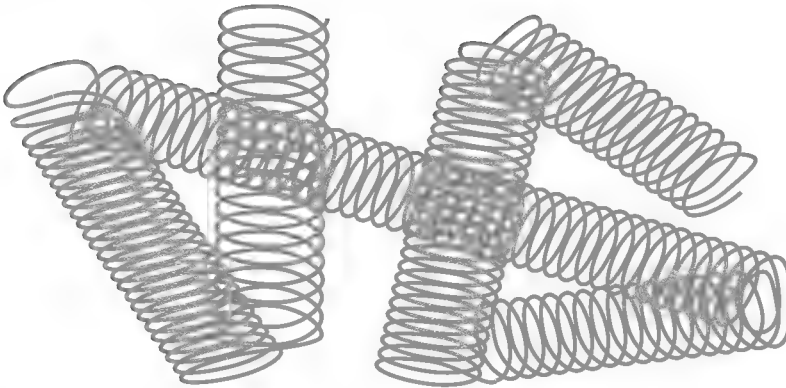
In an alpha helix hydrogen bonds 'pull' molecule chain into a spiral structure



In a beta sheet hydrogen bonds hold and align molecule chains side by side. Angle of bonds in each chain are as a consequence perfectly aligned forming 'pleats'.



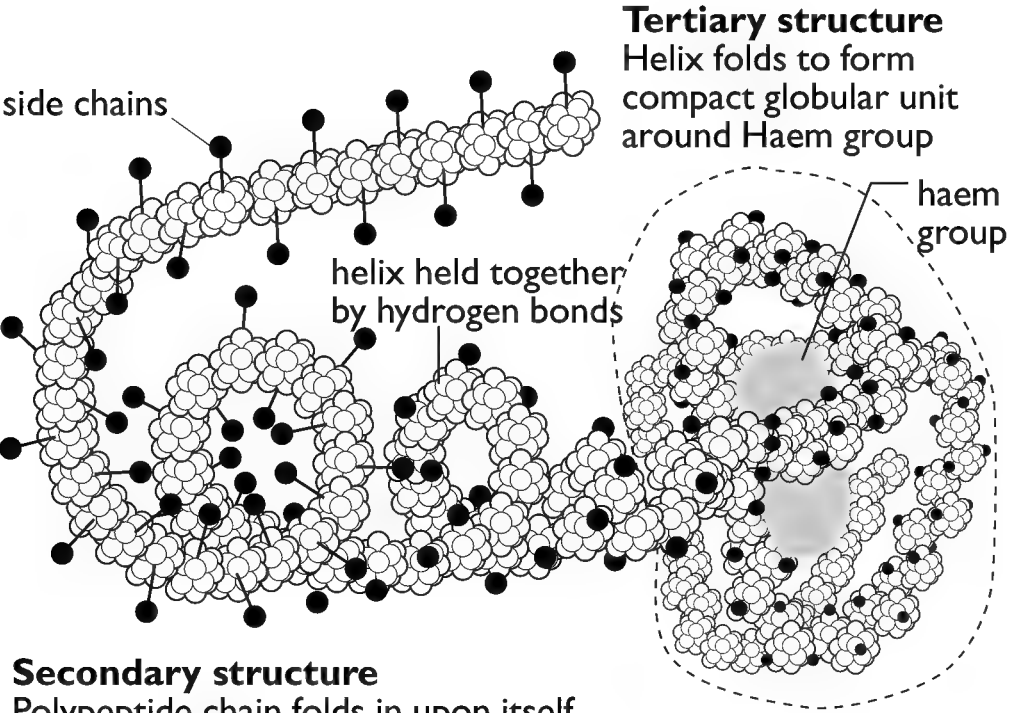
Tertiary structure of proteins



The alpha helix chain folds around itself forming a third level of structural organisation, just like a tangled telephone flex

Primary structure

Amino acids join to form a polypeptide chain



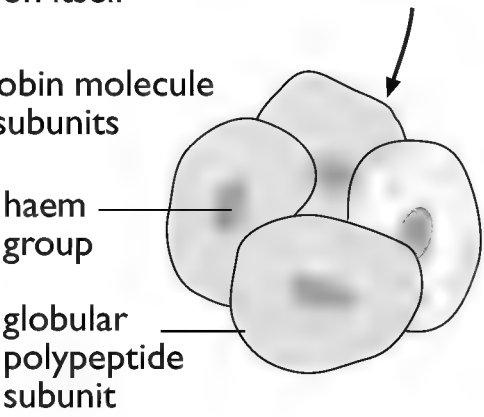
Secondary structure

Polypeptide chain folds in upon itself to form a helix

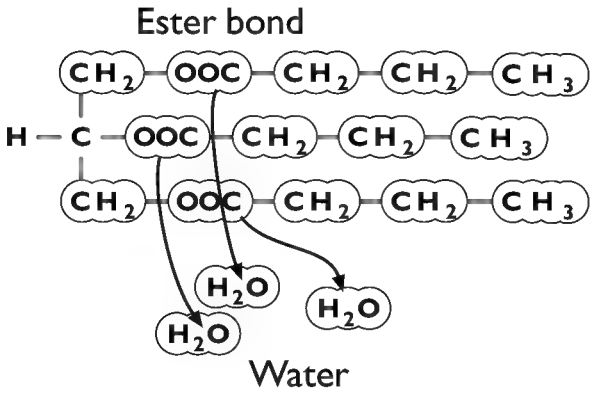
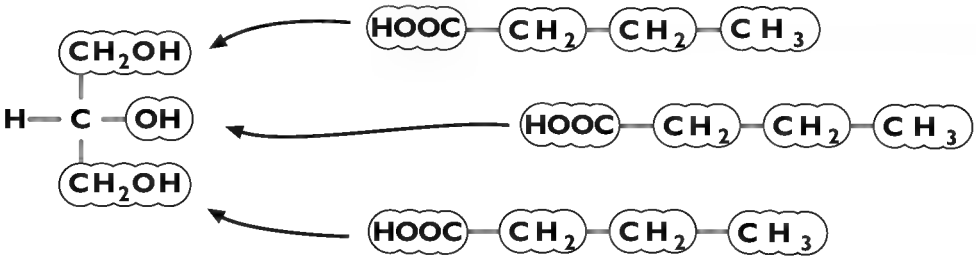
Haemoglobin molecule has four subunits

Quaternary structure

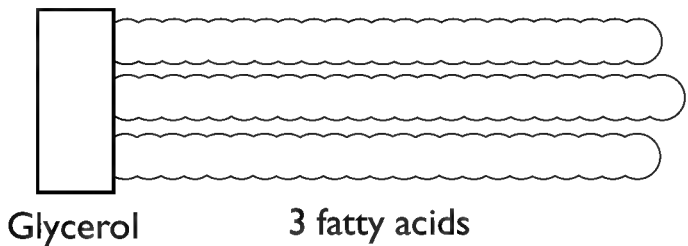
Several polypeptide chain units come together held by disulphide bridges to form a functional protein

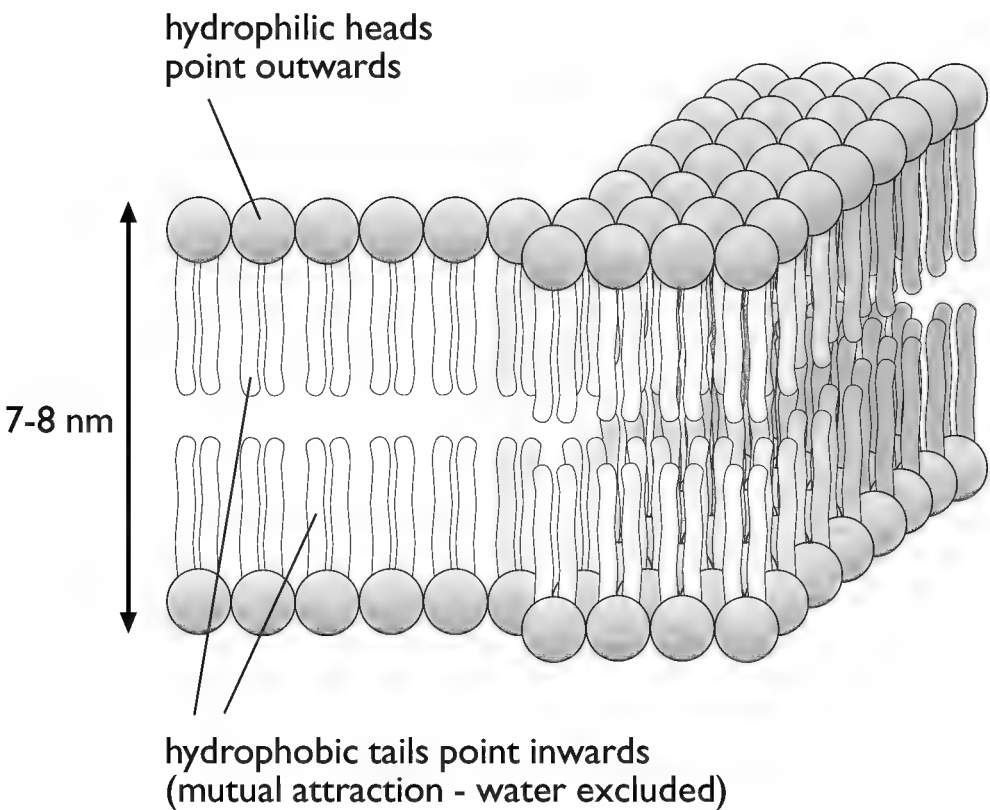


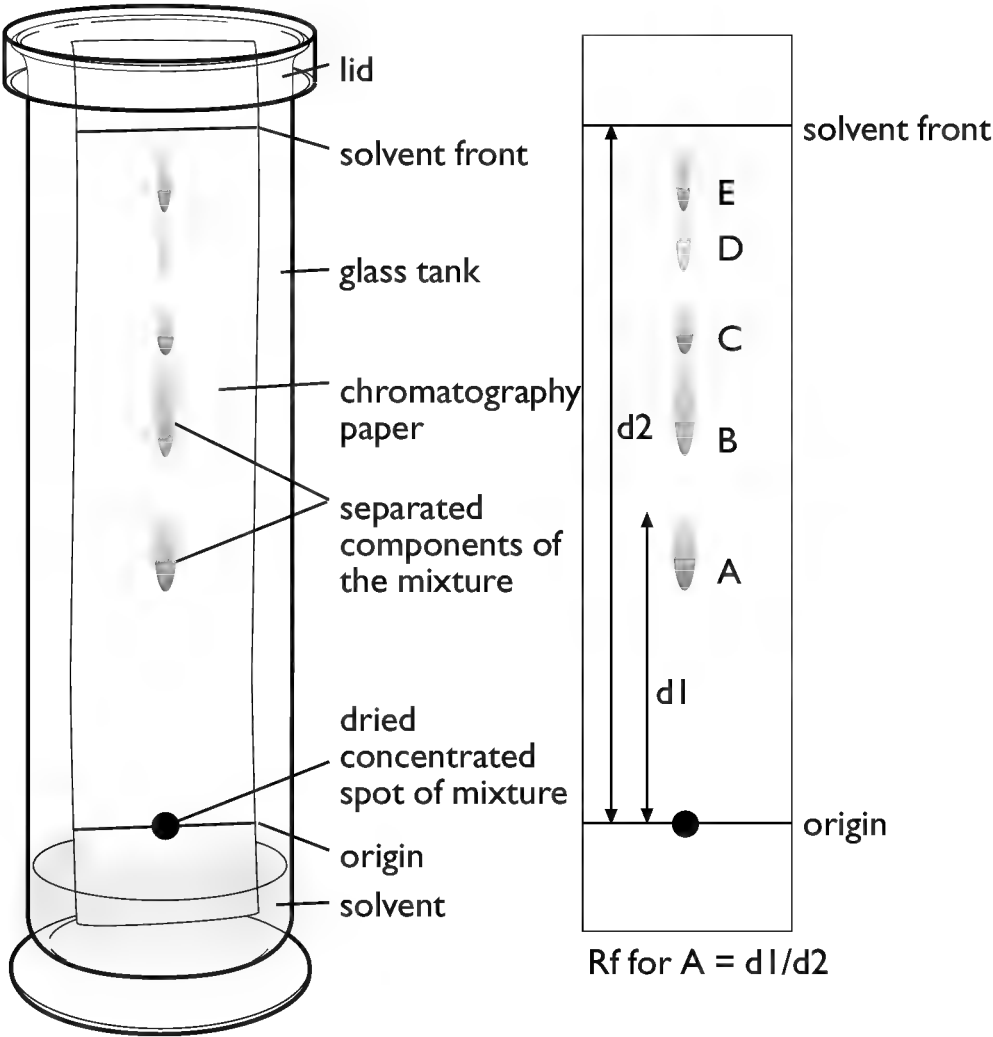
Structure of triglyceride



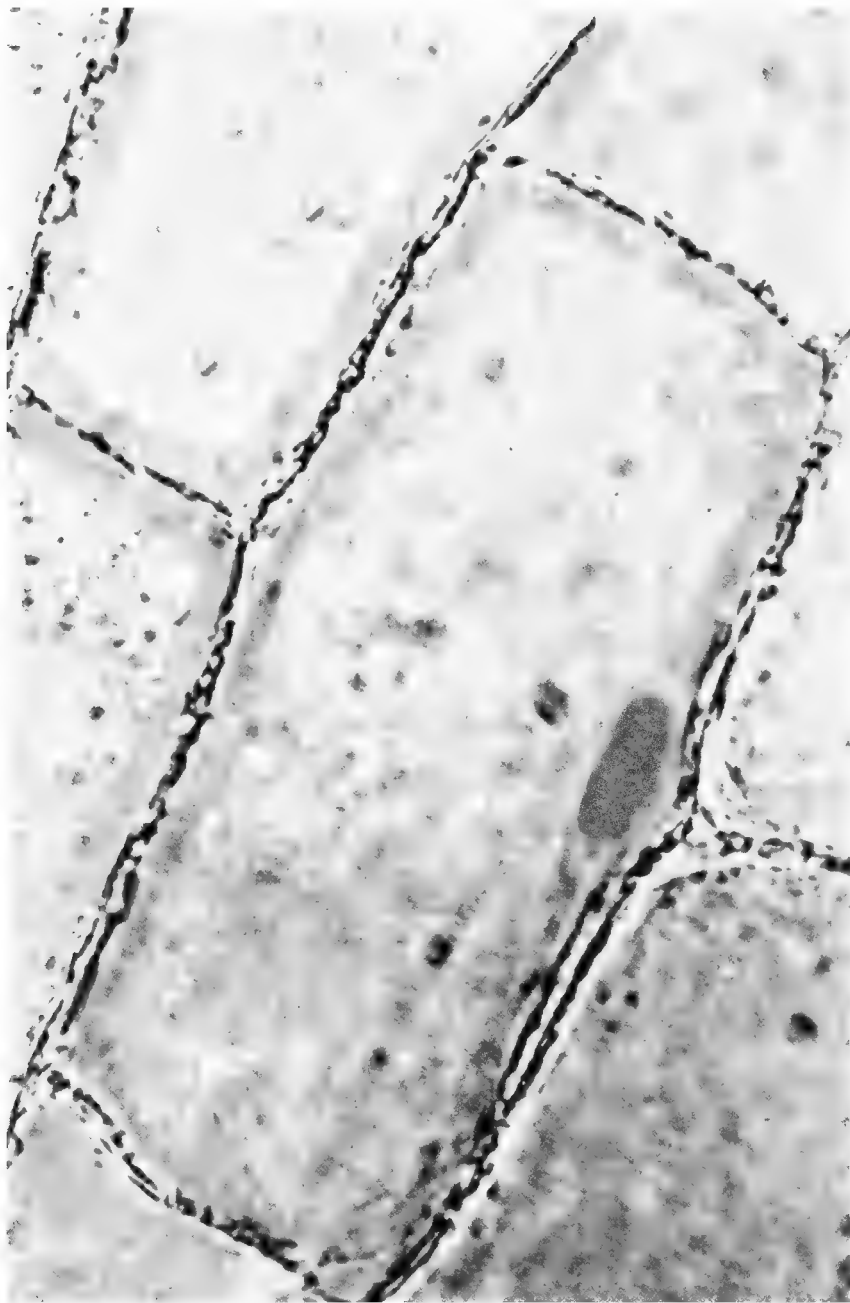
Model of a triglyceride

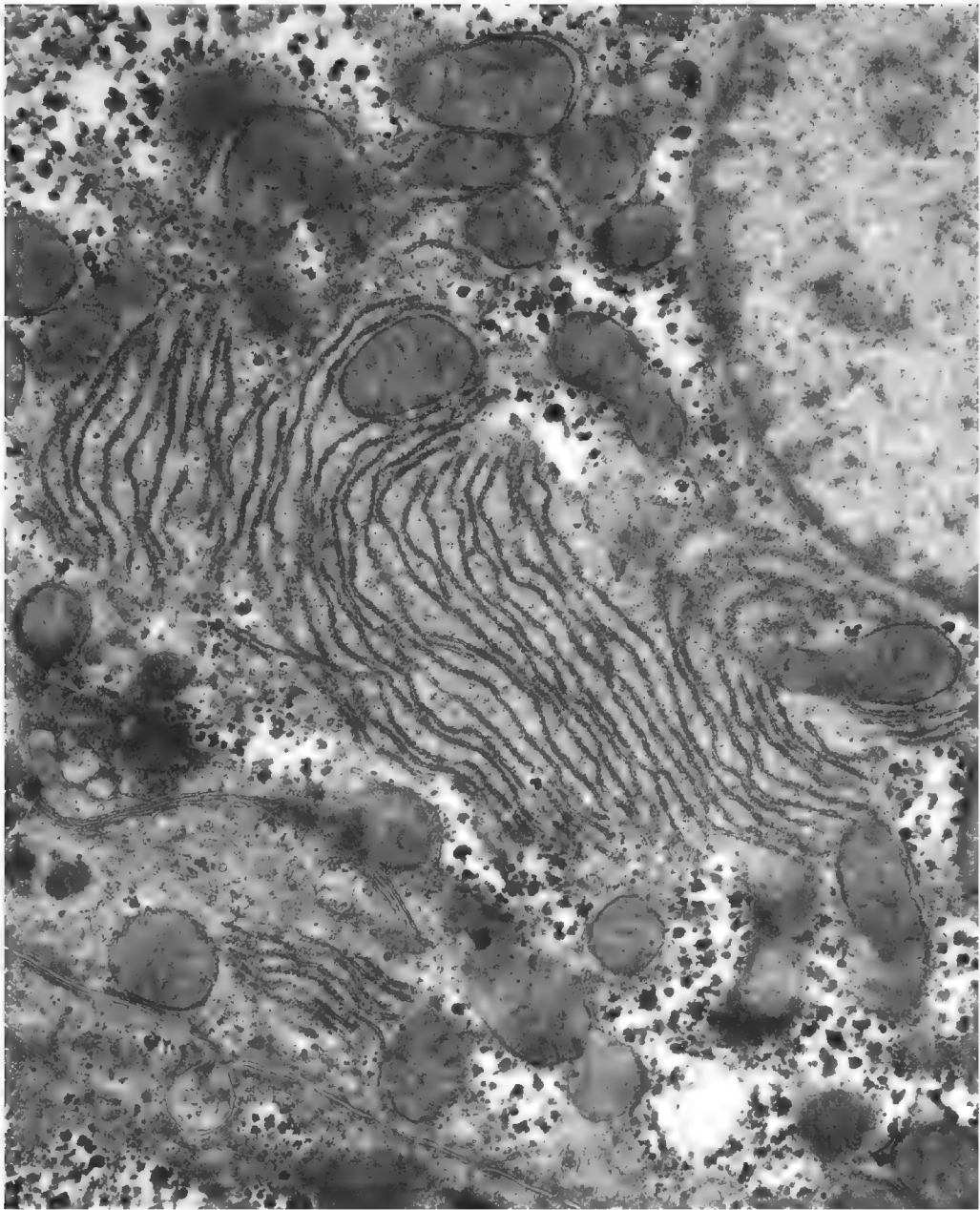


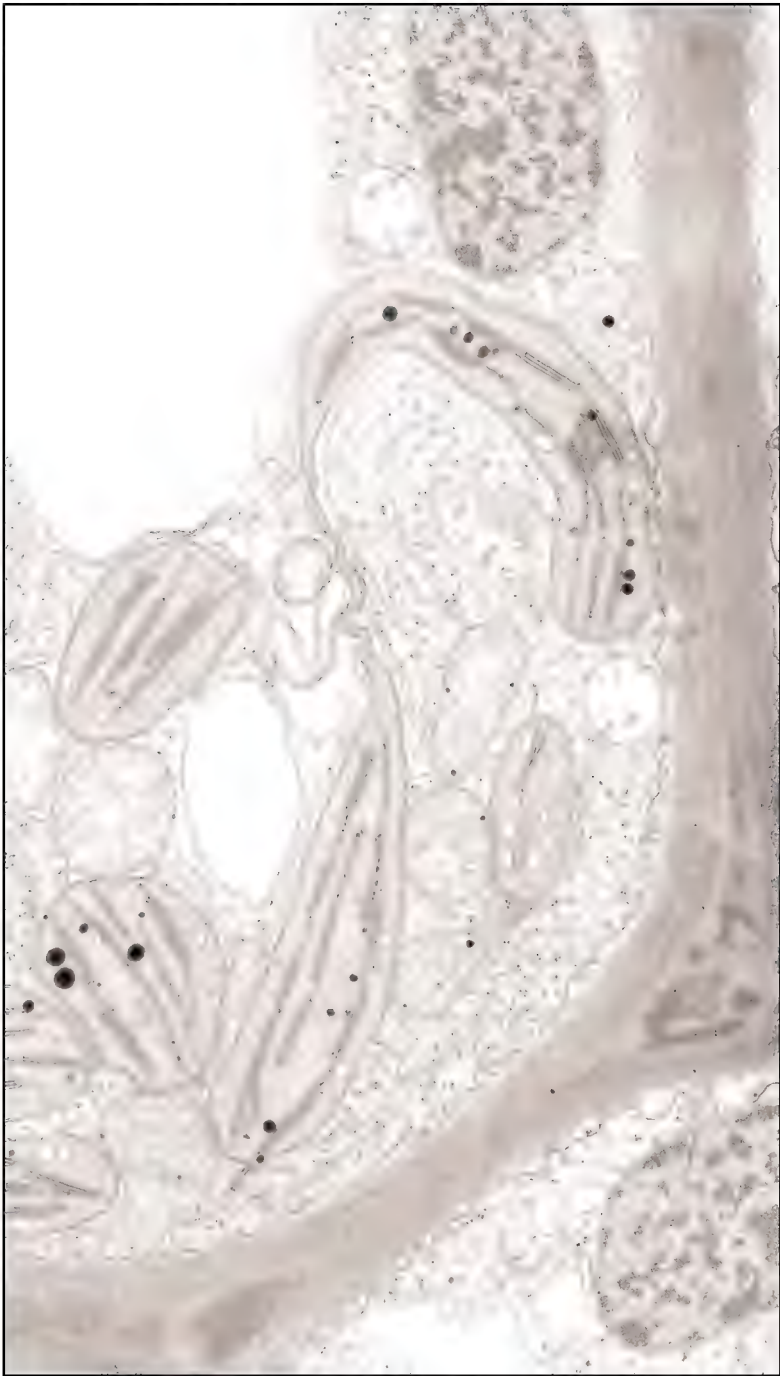


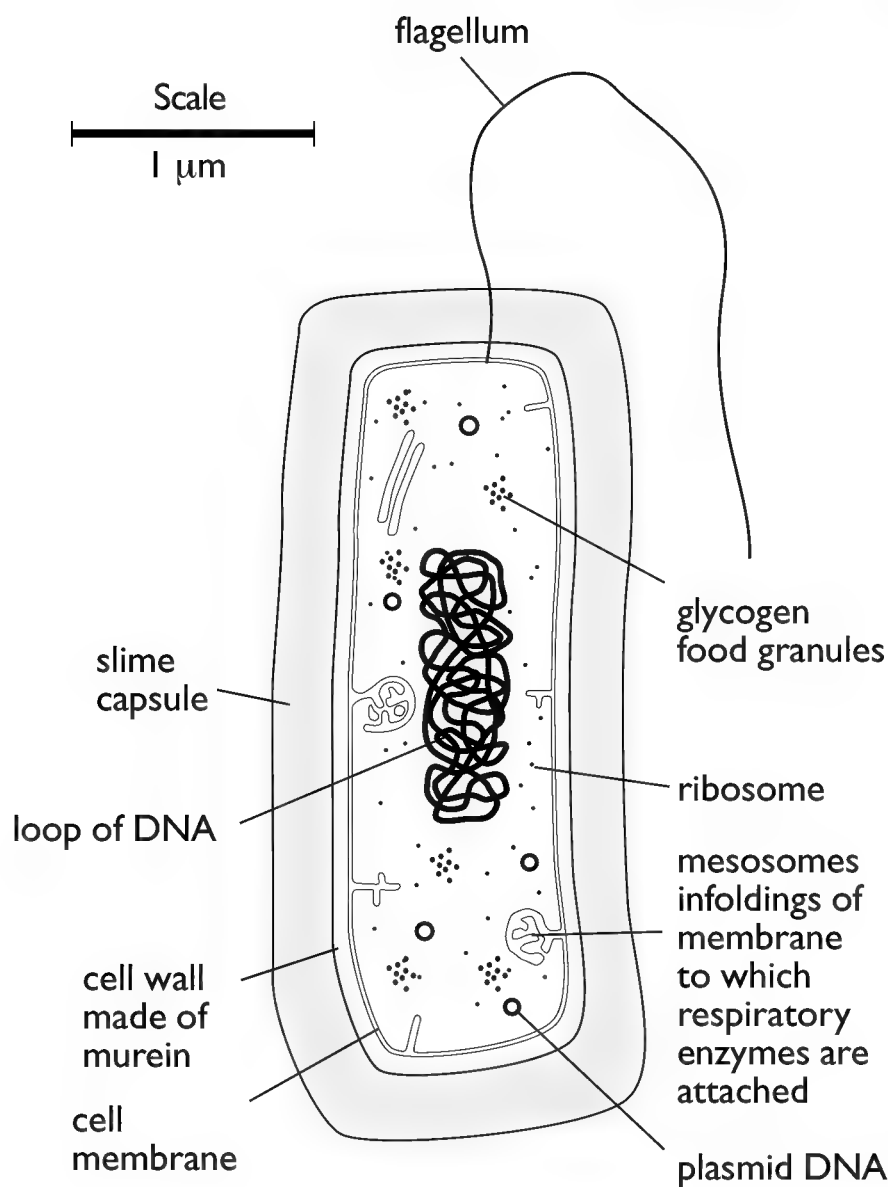




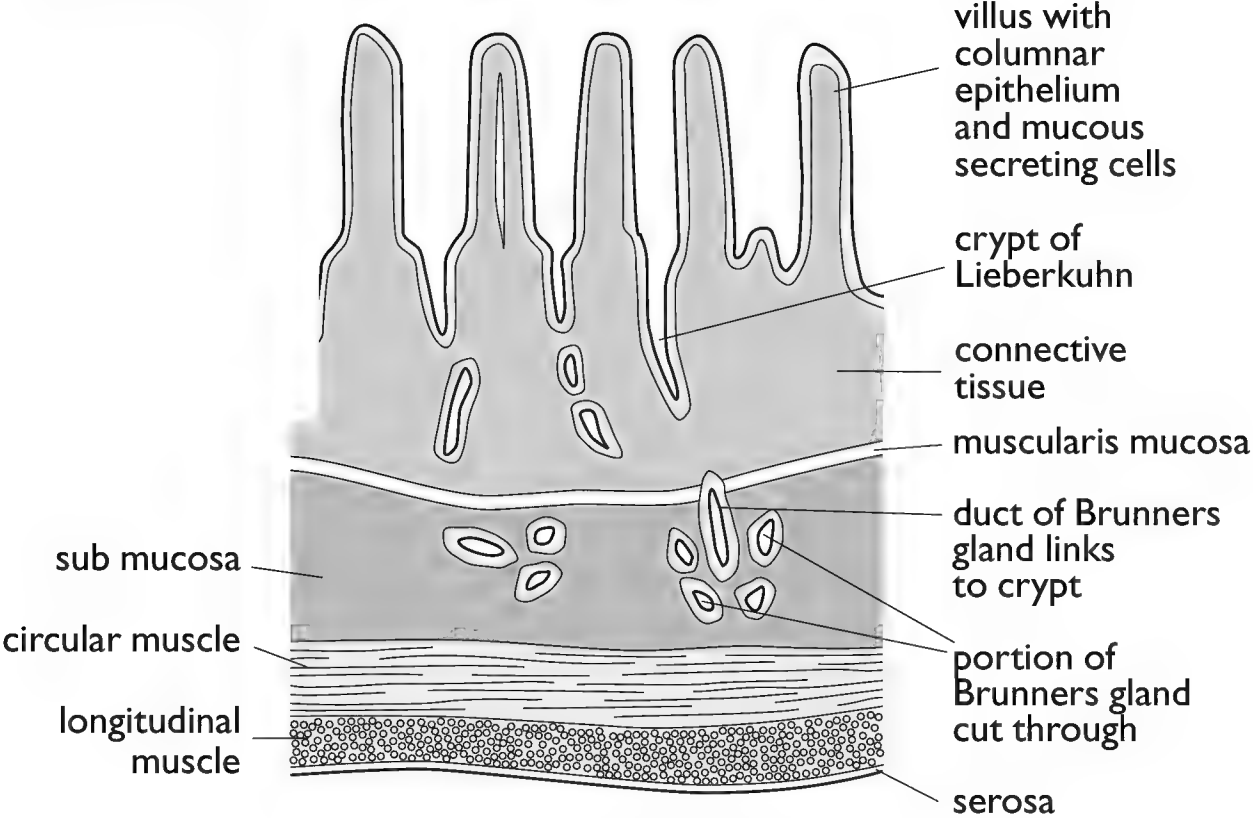


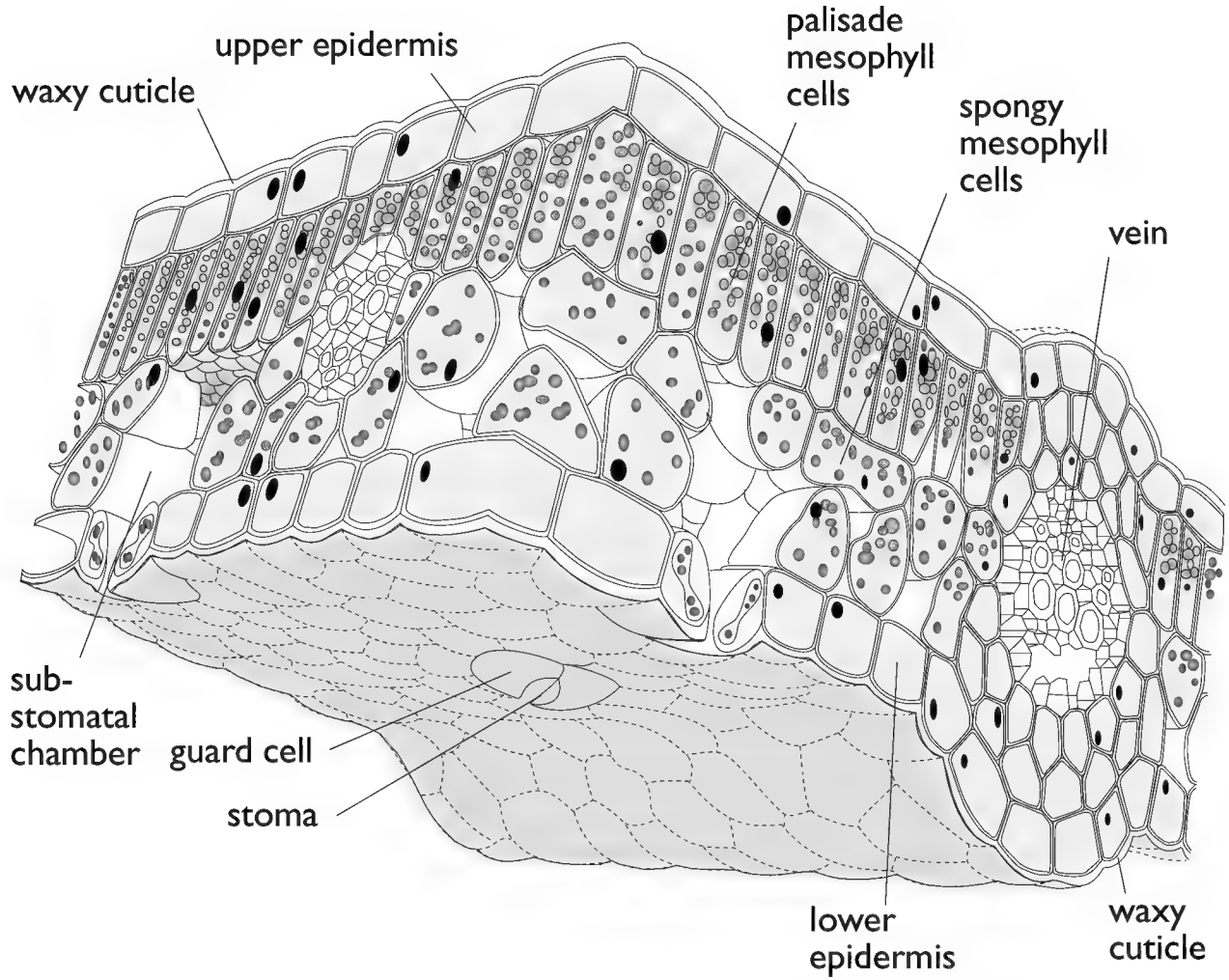


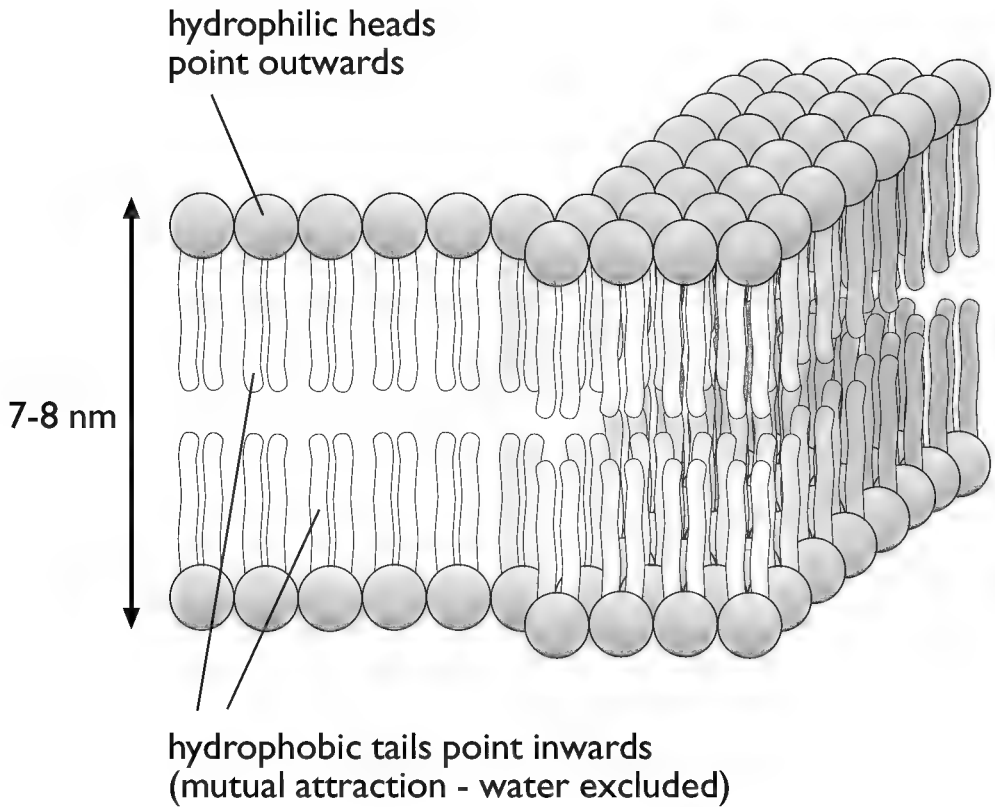


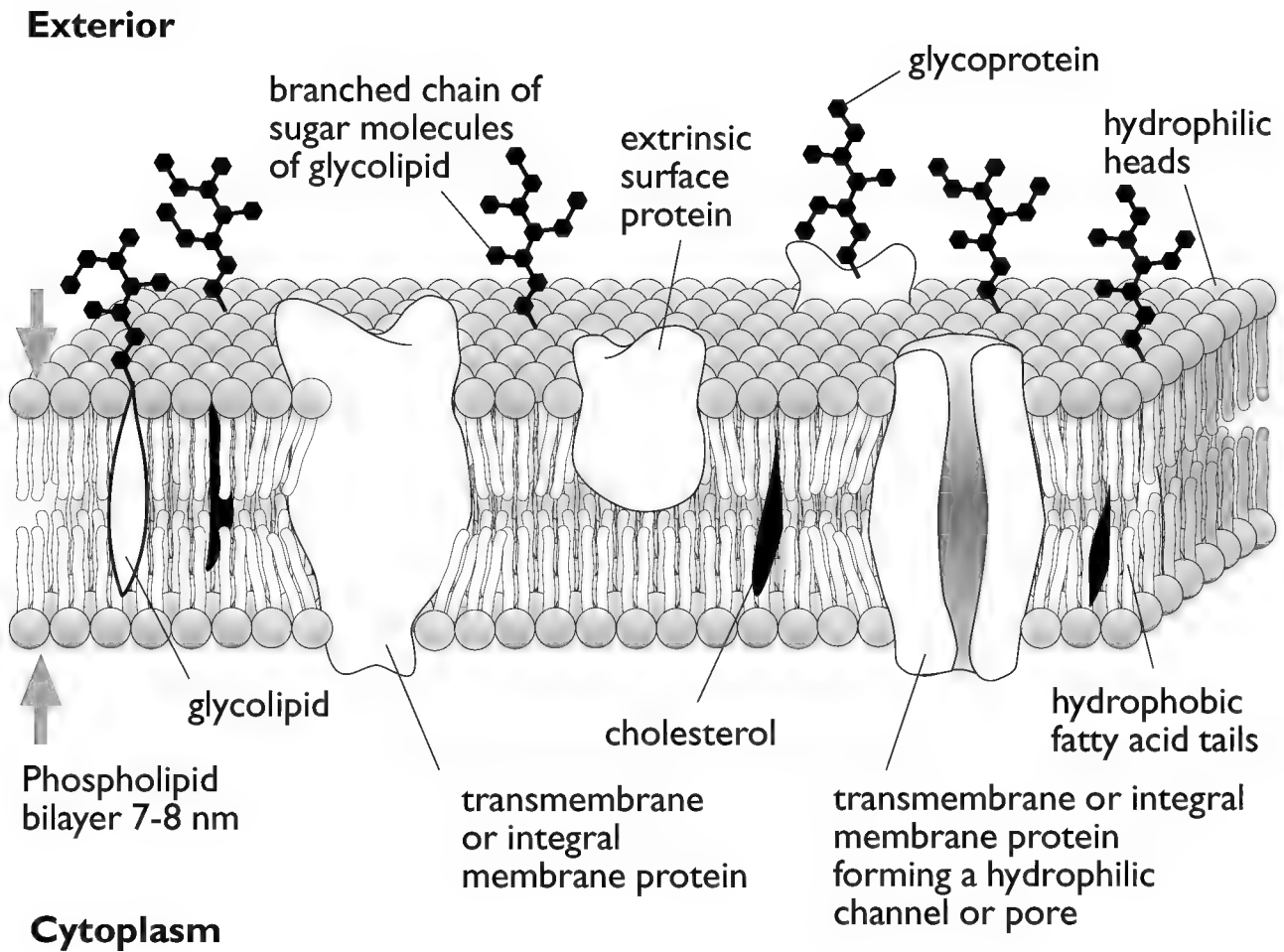


T.S Duodenum





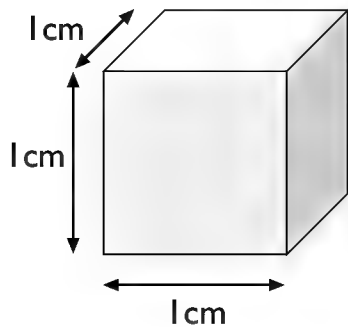




Surface area = 6cm^2

Volume = 1cm^3

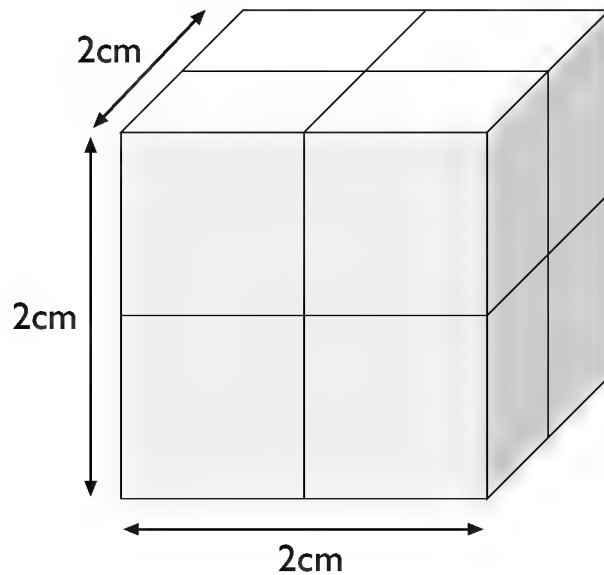
Surface area to volume = $6:1 = 6$

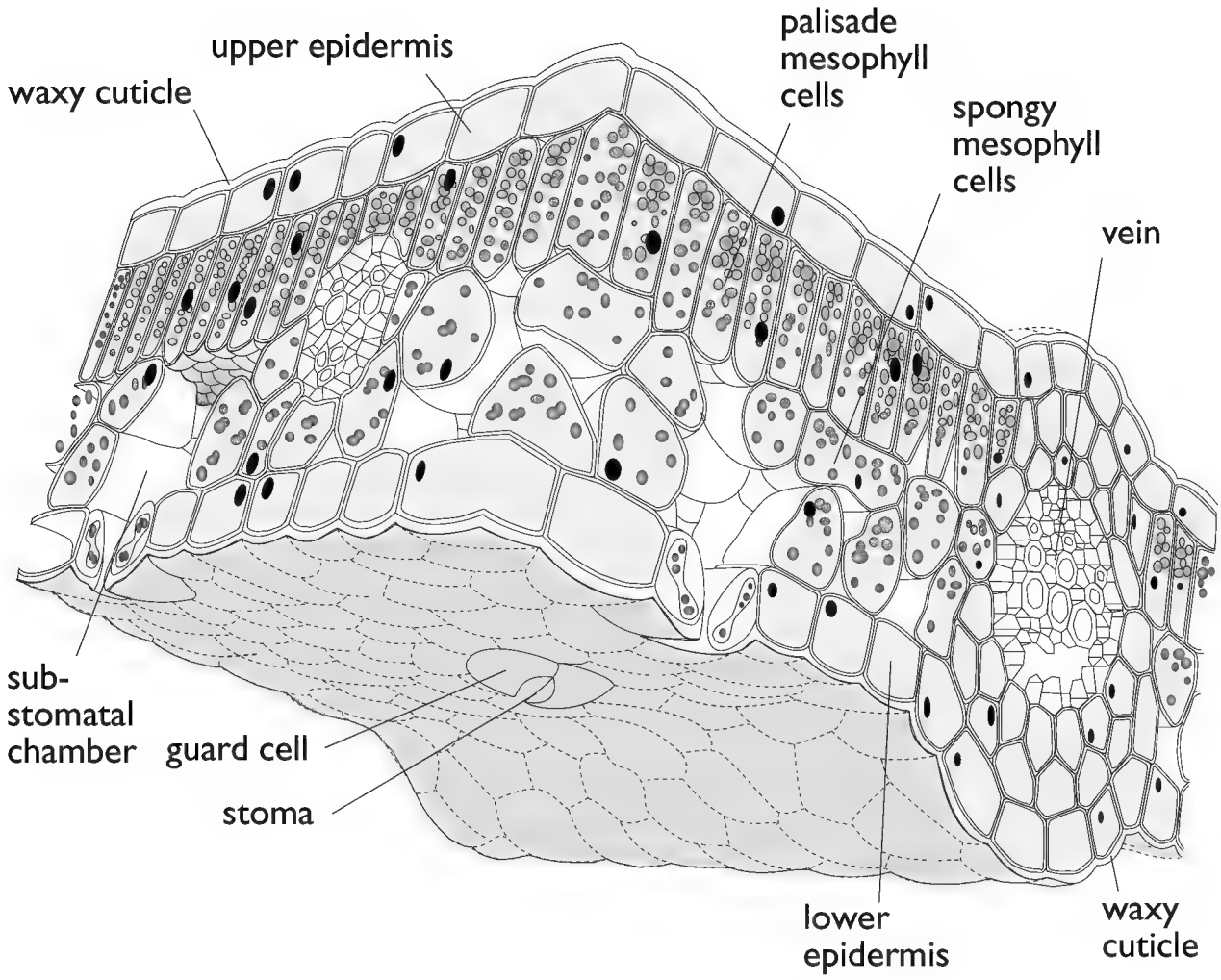


Surface area = 24cm^2

Volume = 8cm^3

Surface area to volume = $24:8 = 3$





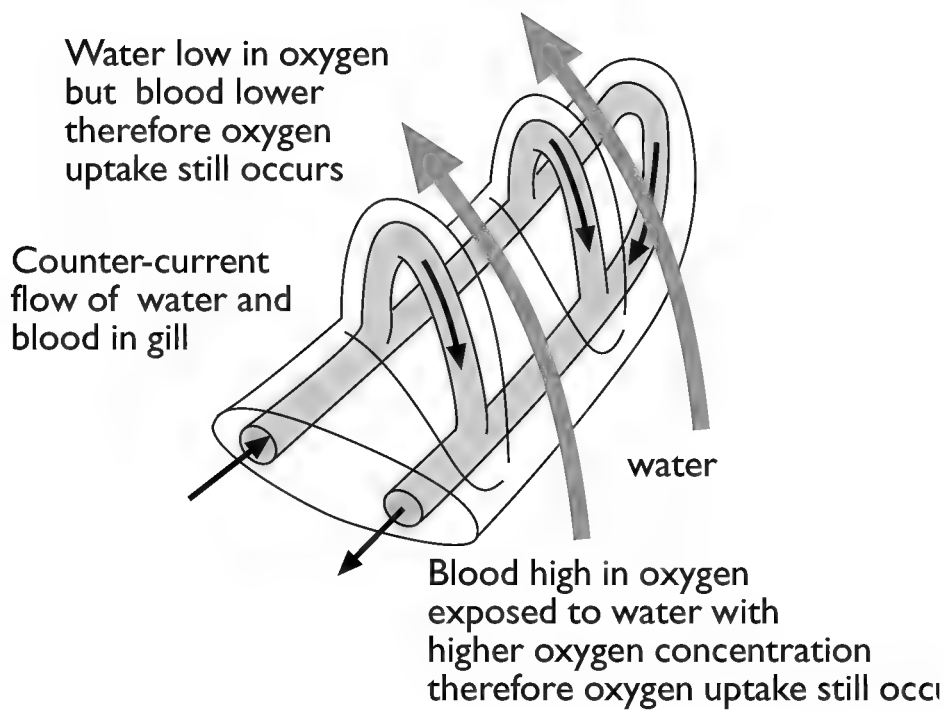
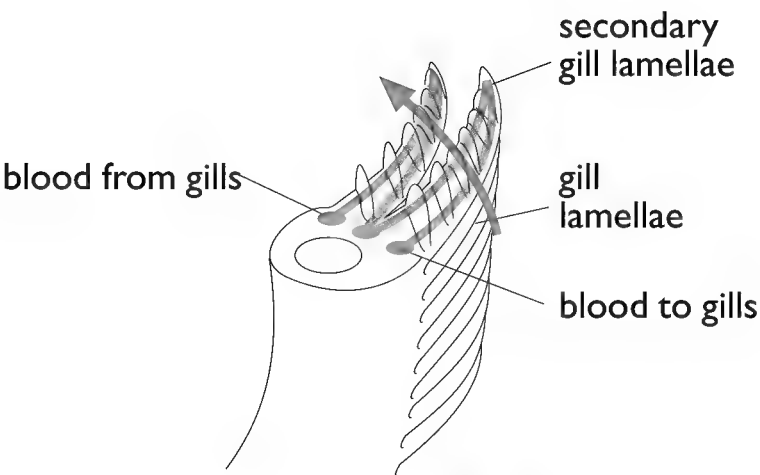
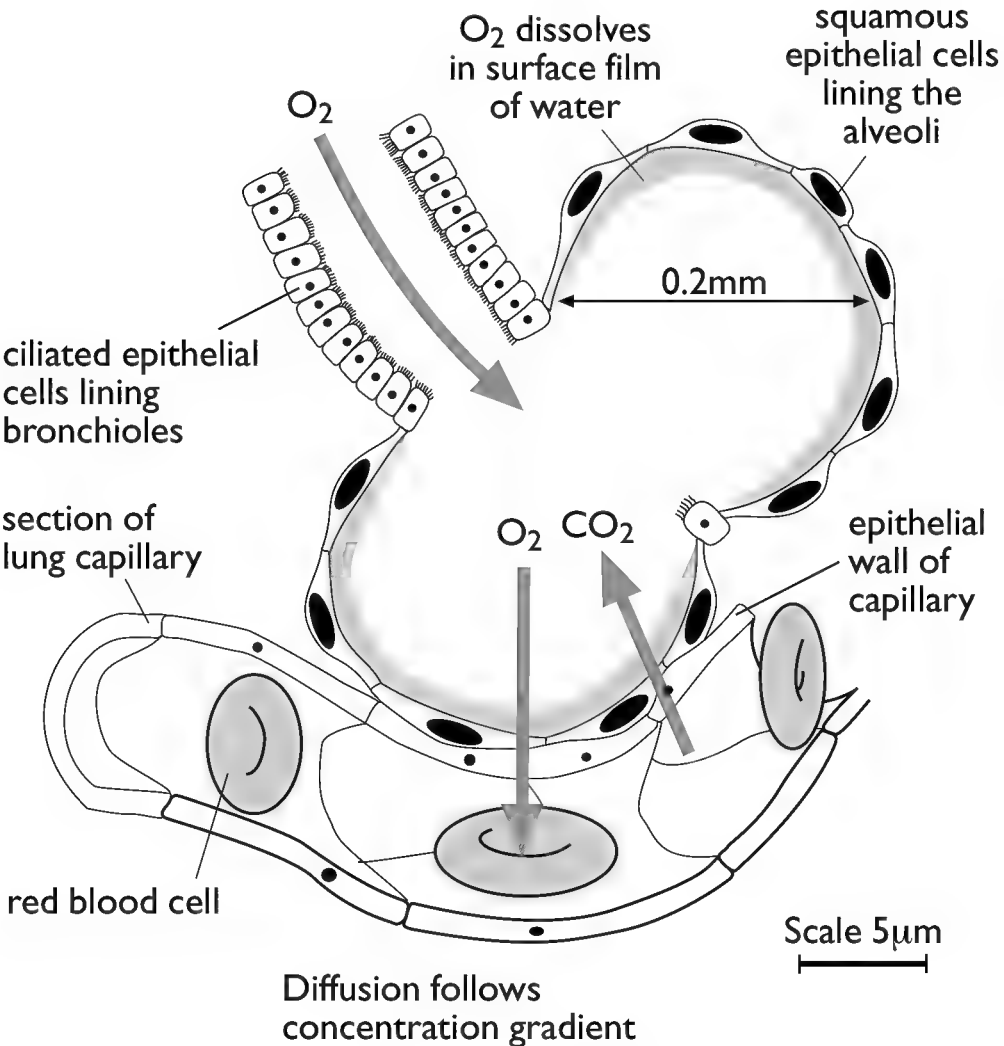


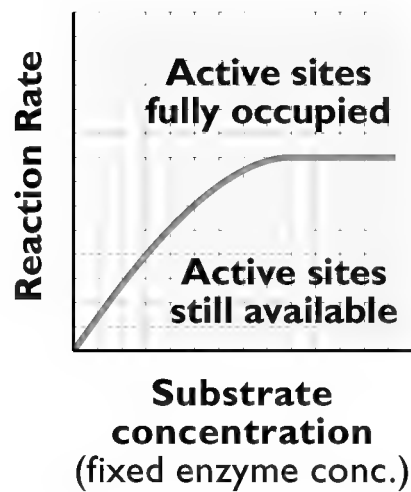
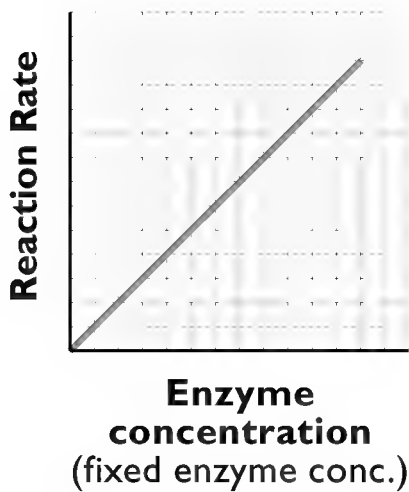
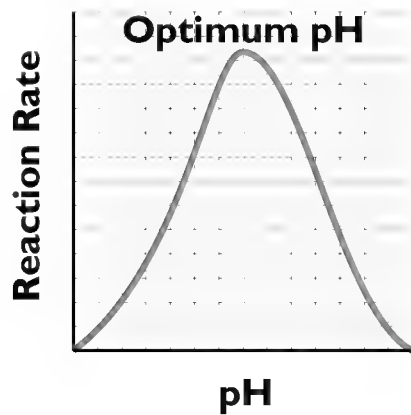
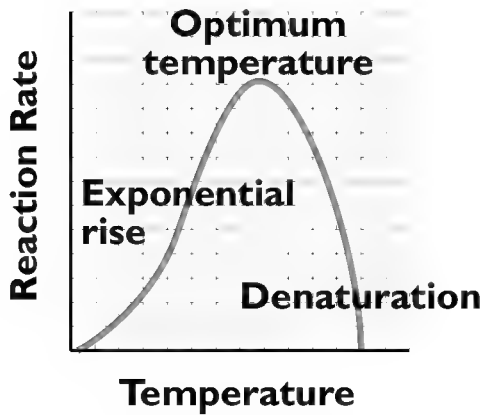
Diagram of Alveoli and Associated Capillaries

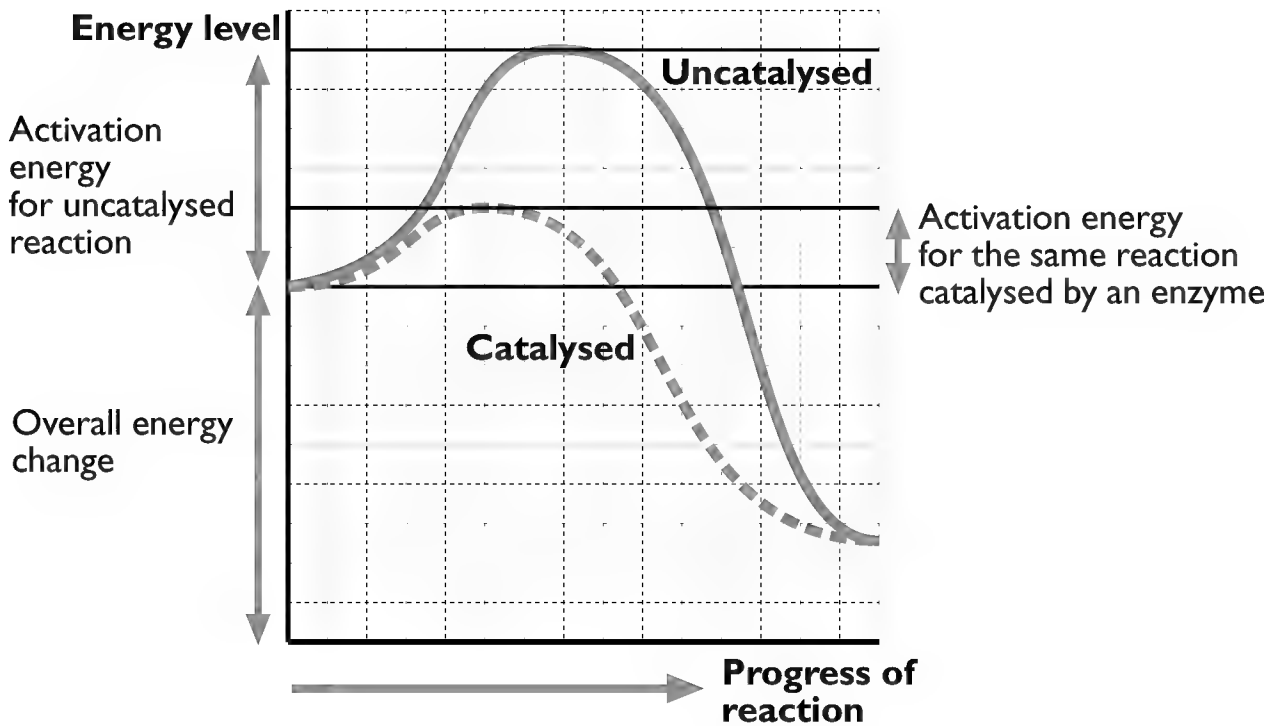
Chapter 11

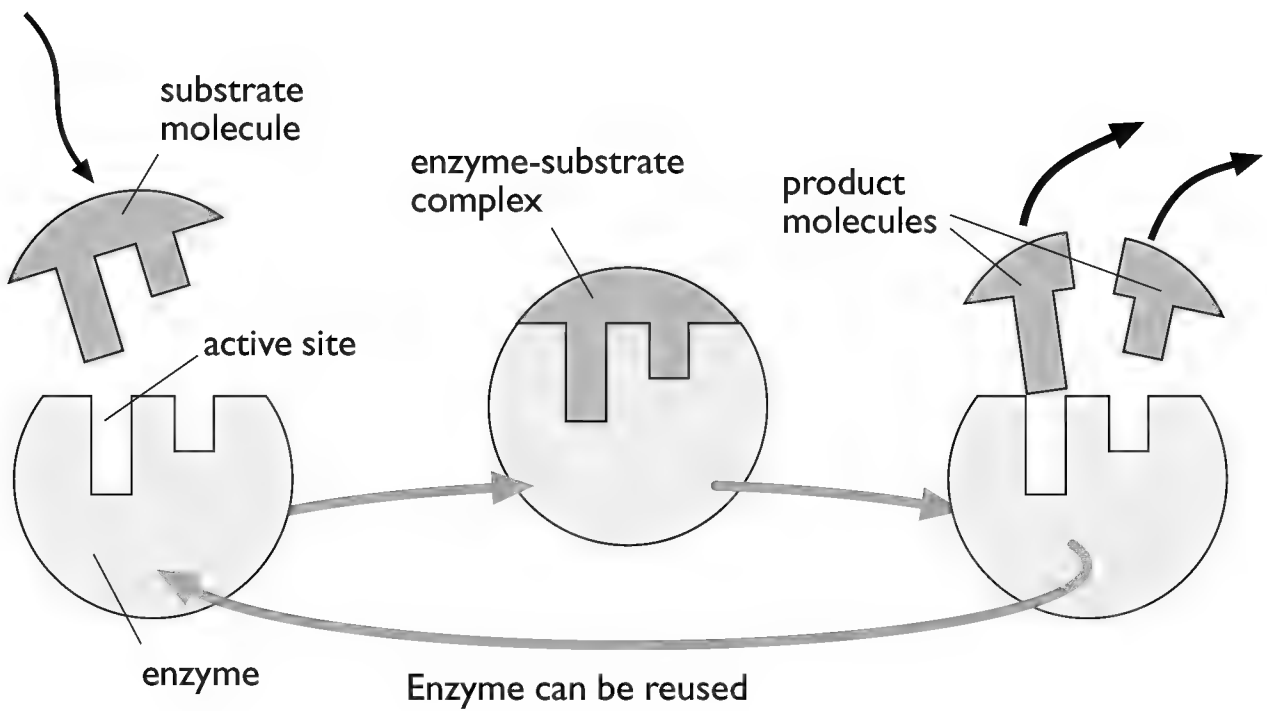


Diffusion distance = approx 2.5µm

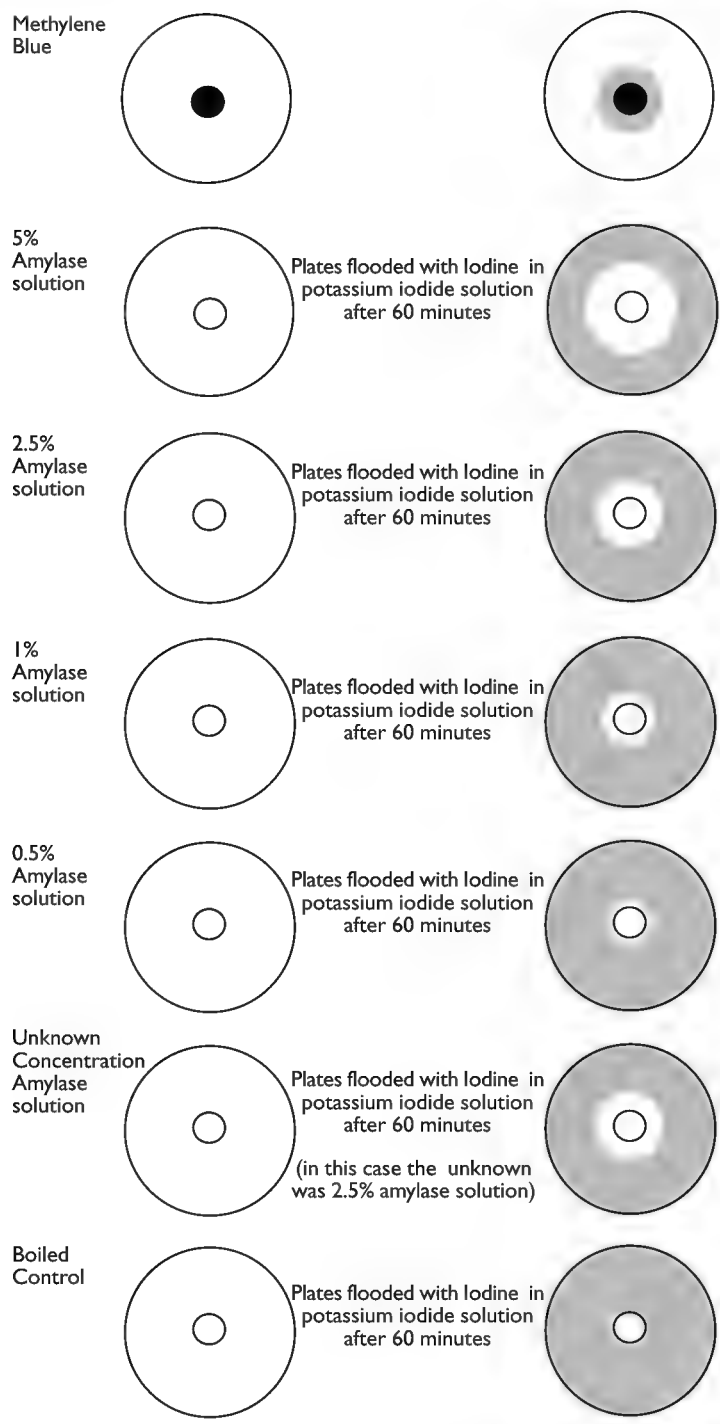
Temperature, pH, Enzyme and Substrate Concentration



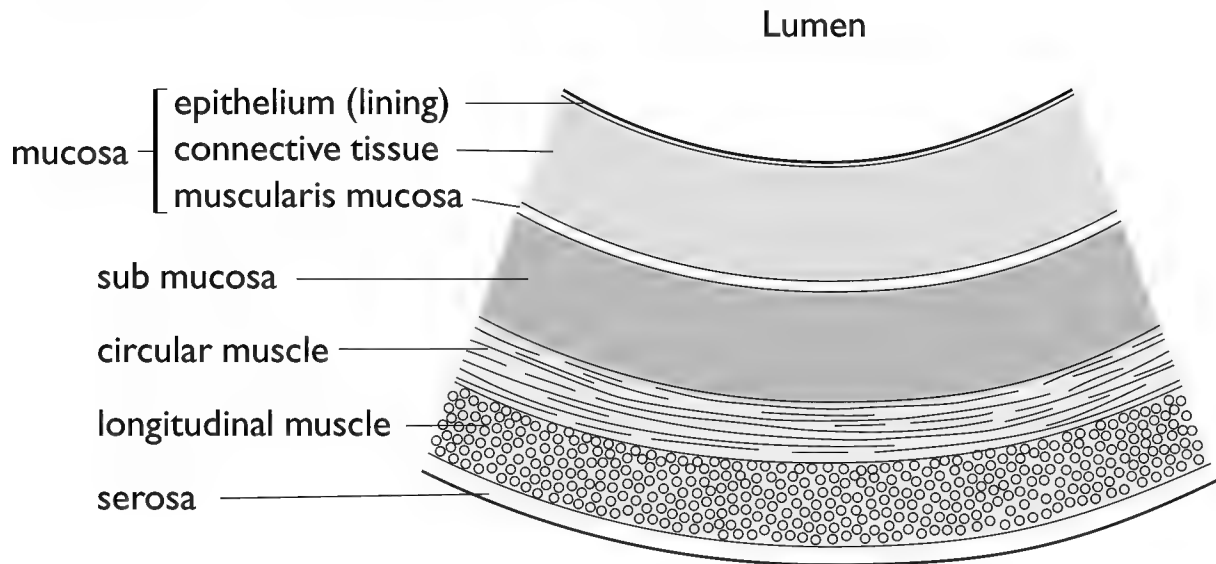




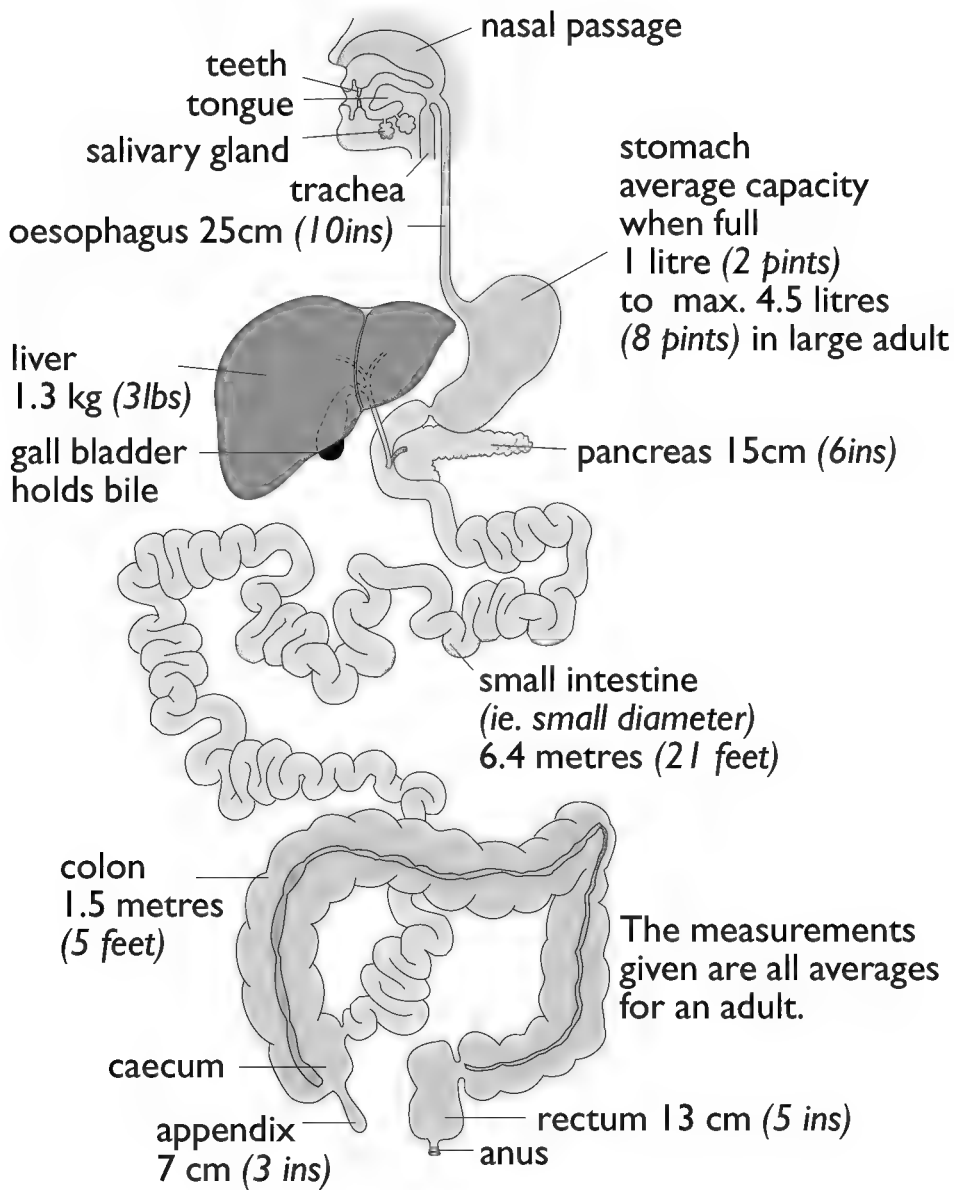
Starch agar plates



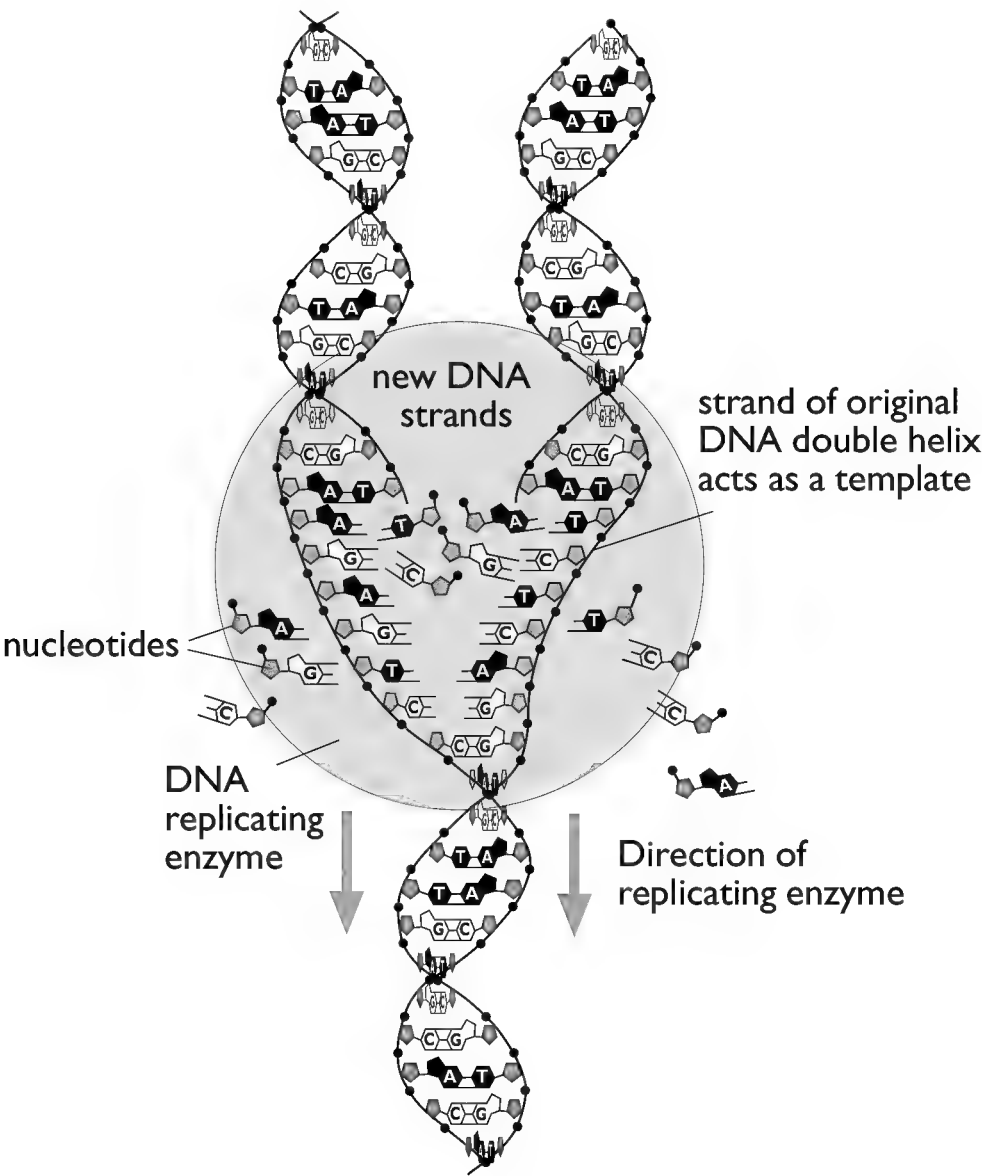
General plan of tissues of alimentary canal



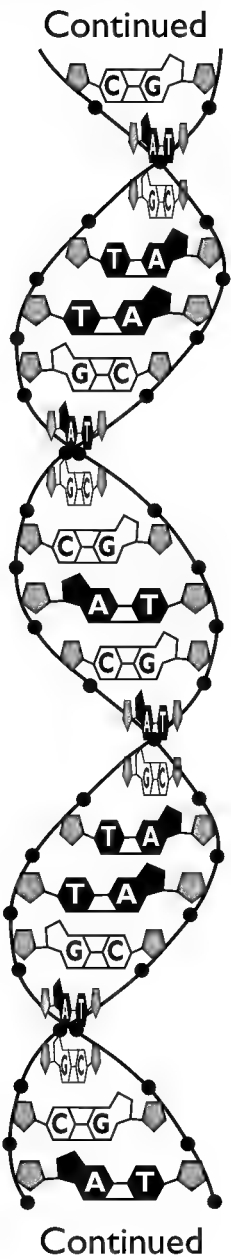
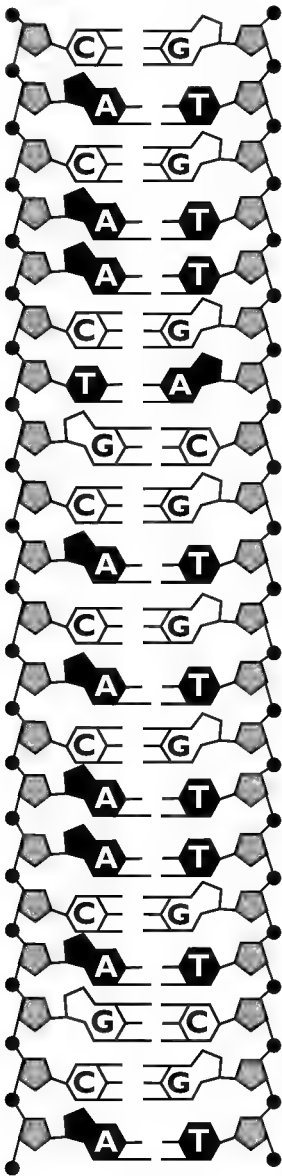
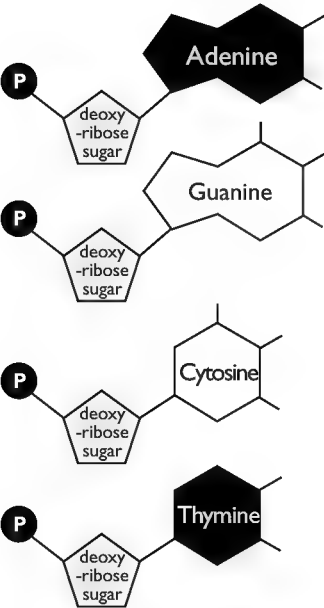
Digestive System Displaced

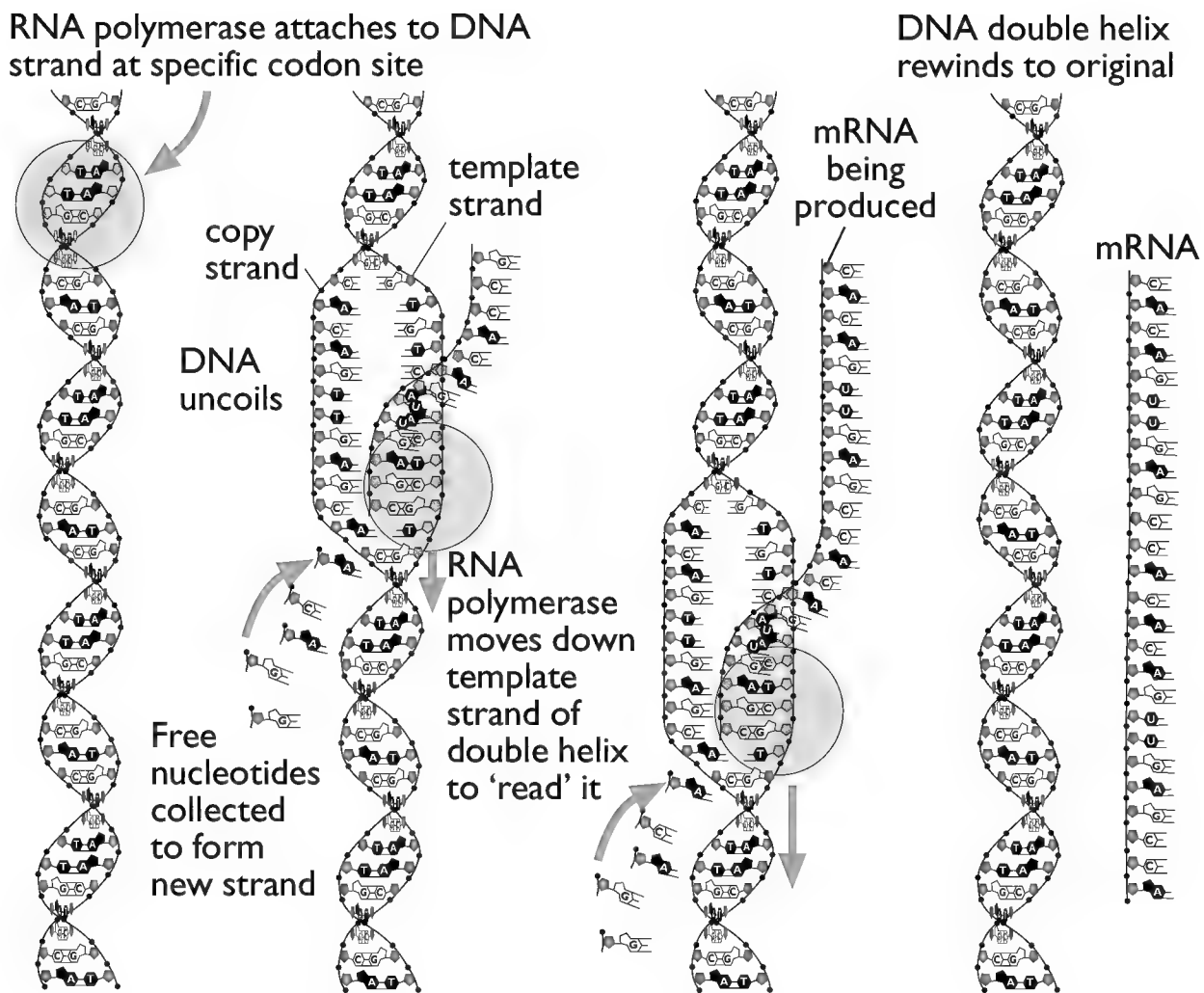


Showing Structure of DNA, RNA and Semi-conservative Replication (with DNA Polymerase)



The Nucleotides of DNA





Translation of mRNA code to build a polypeptide

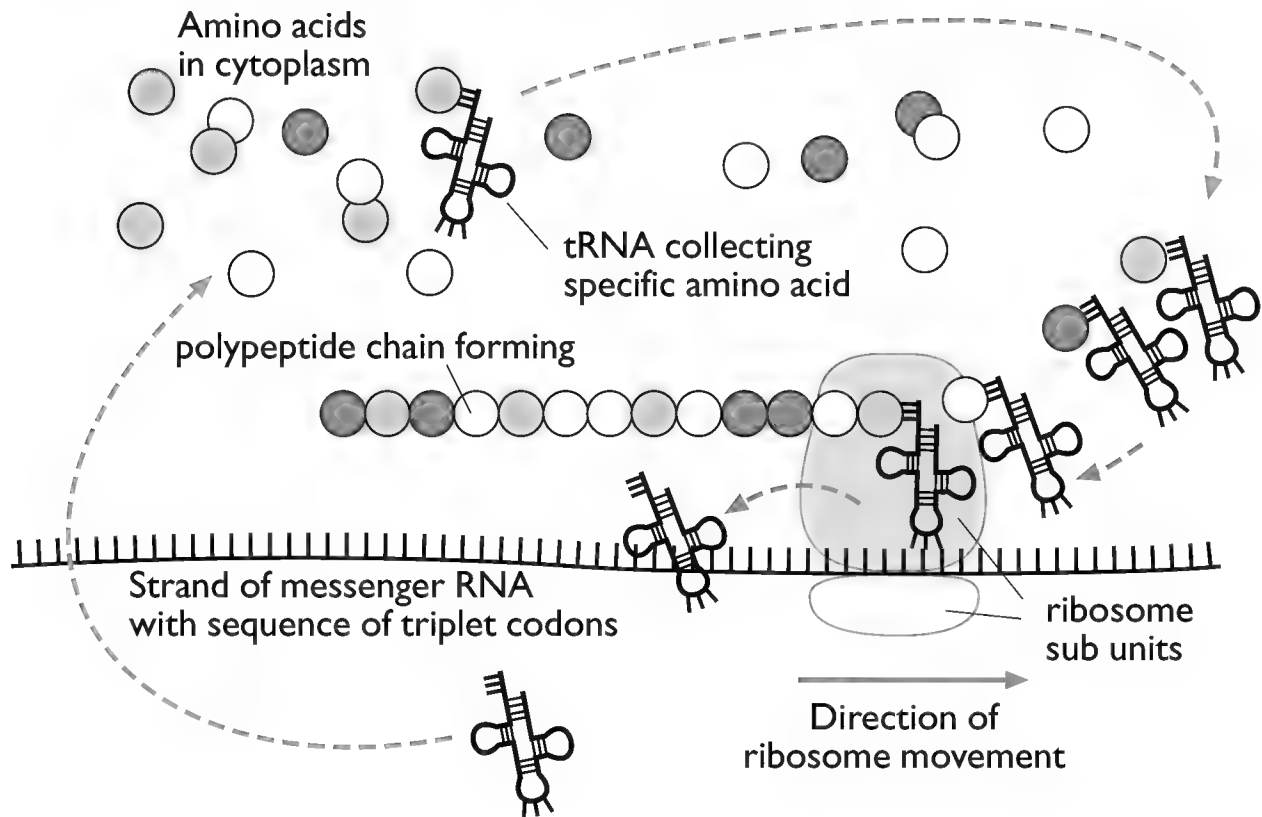


Table of Amino Acid Codons

Amino acid	Abbr.	mRNA codons	Essential
Alanine	Ala	GCA, GCC, GCG, GCU	
Arginine	Arg	AGA, AGG, CGA, CGC, CGG, CGU	infants only
Asparagine	Asn	AAC, AAU	
Aspartic acid	Asp	GAC, GAU	
Cysteine	Cys	UGC, UGU	
Glutamic acid	Glu	GAA,GAG	
Glutamine	Gln	CAA, CAG	
Glycine	Gly	GGA, GGC, GGG, GGU	
Histidine	His	CAC, CAU	infants only
Isoleucine	Ile	AUA,AUC, AUU	E
Leucine	Leu	CUA, CUC, CUG, CUU, UUA, UUG	E
Lysine	Lys	AAA, AAG	E
Methionine	Met	AUG (Start codon)	E
Phenylalanine	Phe	UUC, UUU	E
Proline	Pro	CCA, CCC, CCG, CCU	
Serine	Ser	AGC, AGU, UCA, UCC, UCG, UCU	
Threonine	Thr	ACA, ACC, ACG, ACU	E
Tryptophan	Trp	UGG	E
Tyrosine	Tyr	UAC, UAU	
Valine	Val	GUA, GUC, GUG, GUU	E
STOP CODONS		UAA, UAG, UGA	

Deletions

C - G - G - C - T - A - T - T - A - A - G - G

C - G - G - C - T - T - T - A - A - G - G

Additions

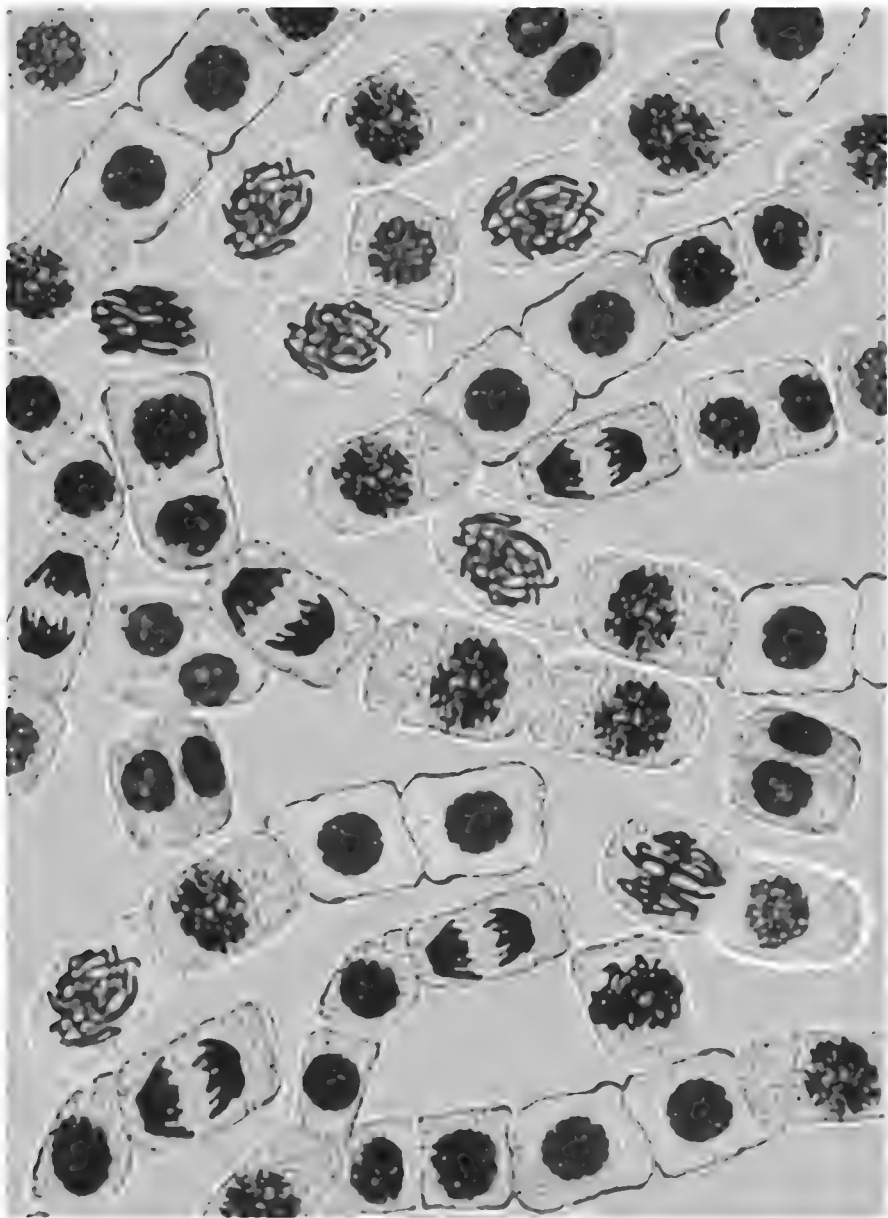
C - G - G - C - T - A - T - T - A - A - G - G

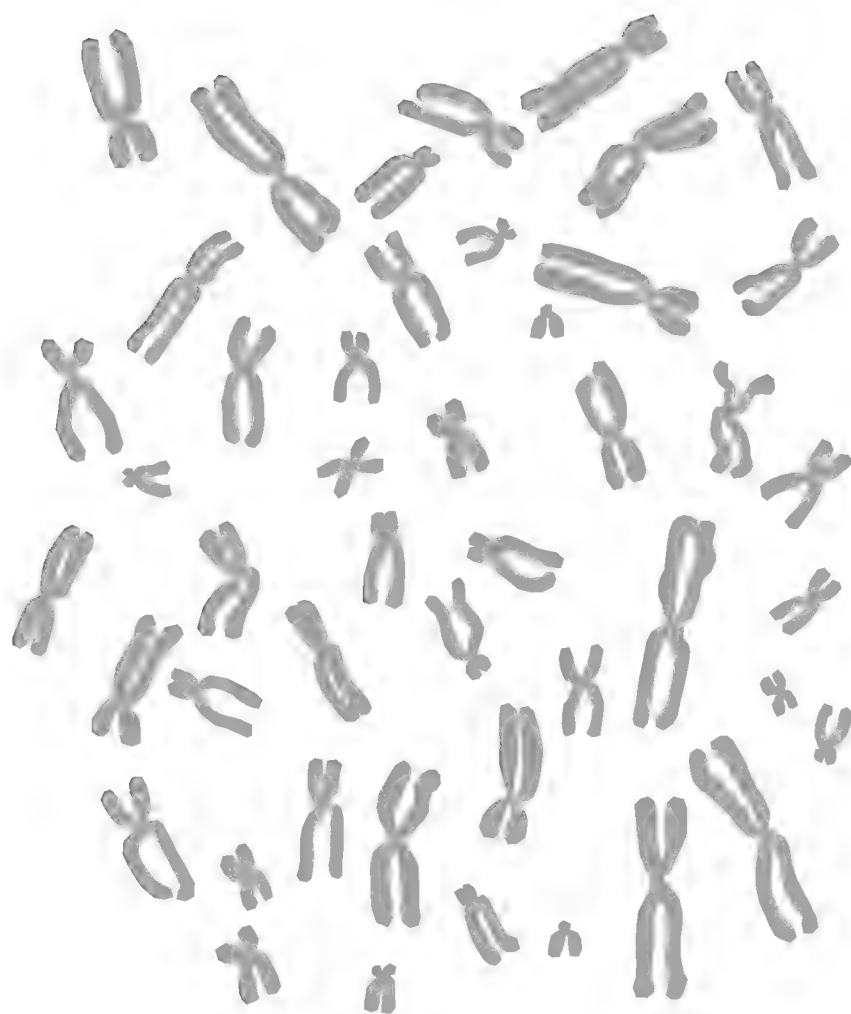
C - G - G - C - T - A - G - T - T - A - A - G - G

Substitutions

C - G - G - C - T - A - T - T - A - A - G - G

C - G - G - C - A - A - T - T - A - A - G - G

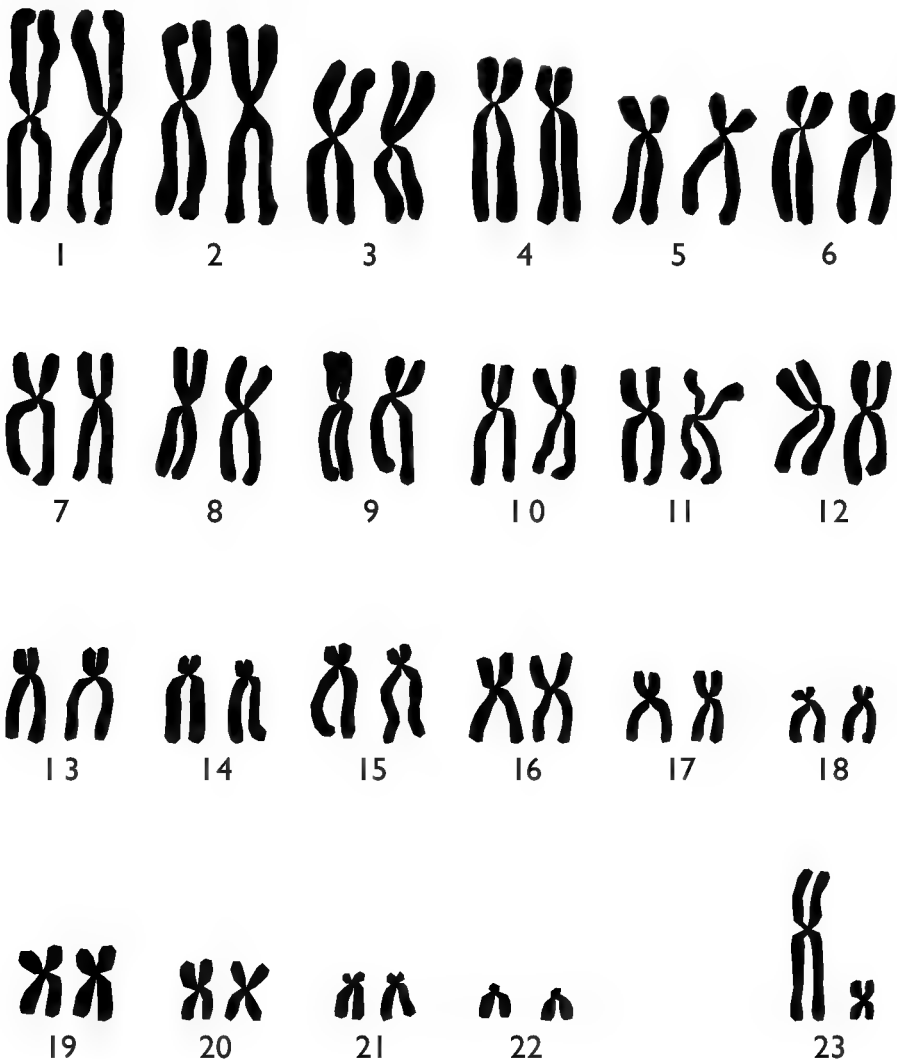


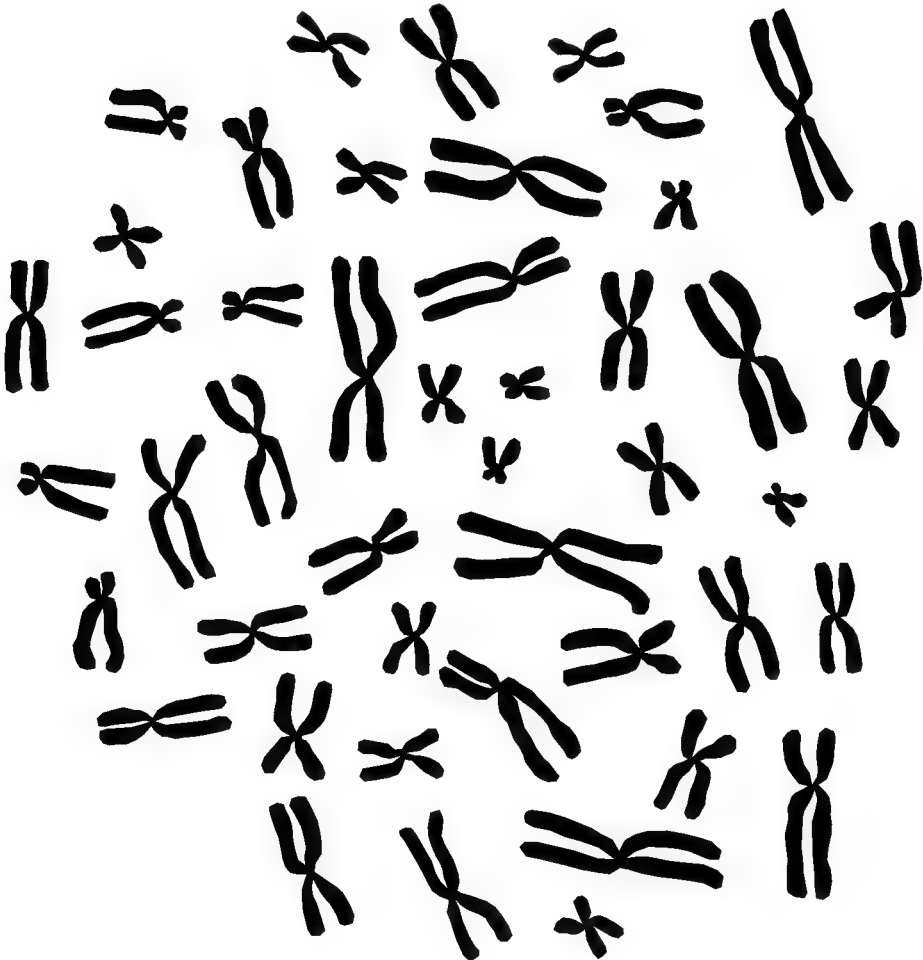


Normal Human Karyotype analysed into homologous pairs



Normal Human Male

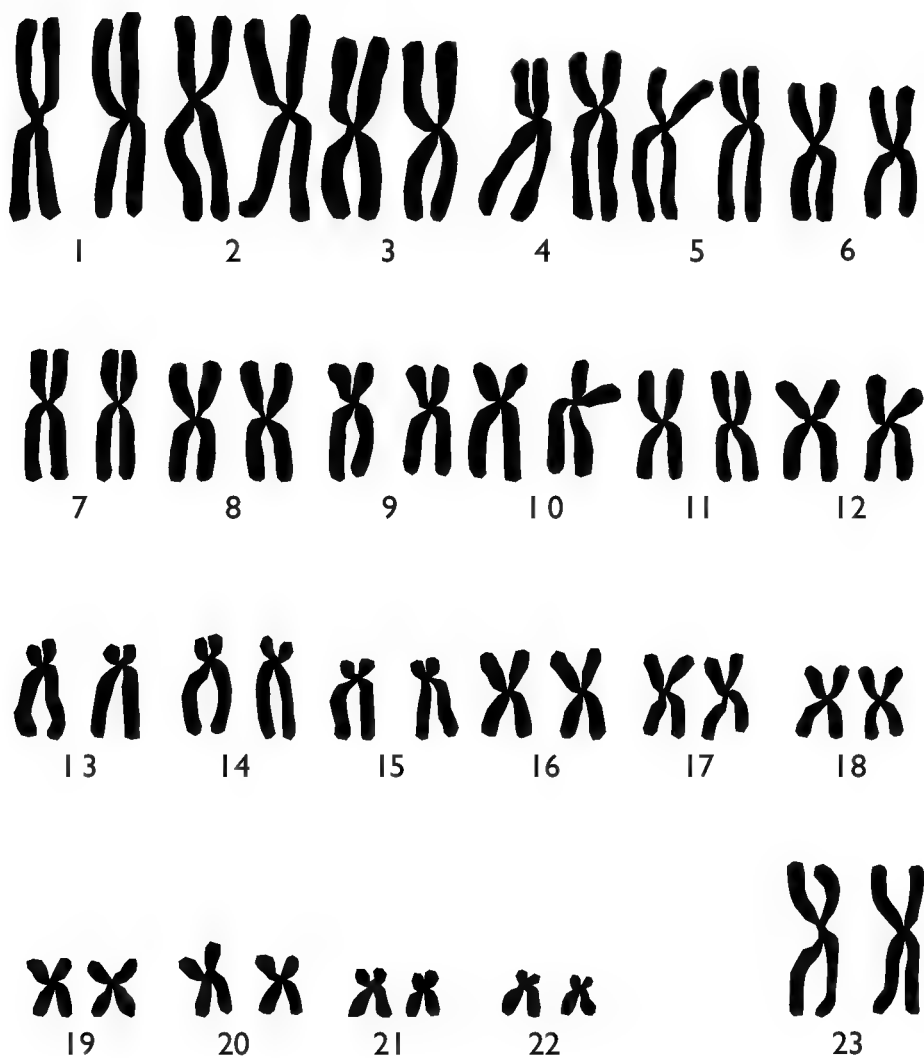




Normal Human Karyotype analysed into homologous pairs

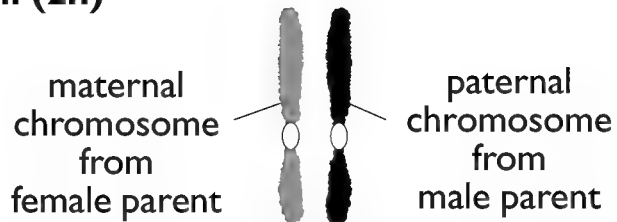


Normal Human Female

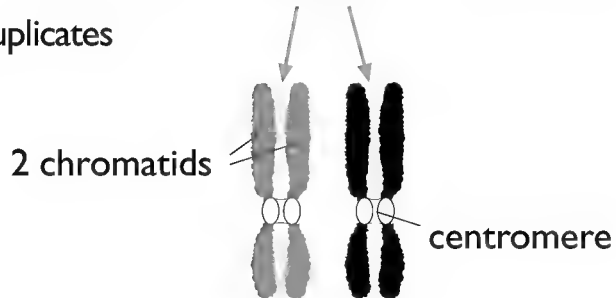


Diploid Parent Cell (2n)

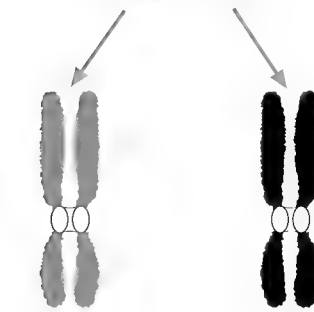
Only one pair of homologous chromosomes are shown



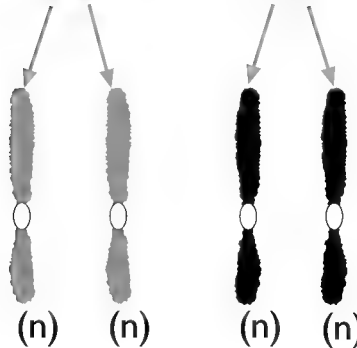
Each chromosome duplicates



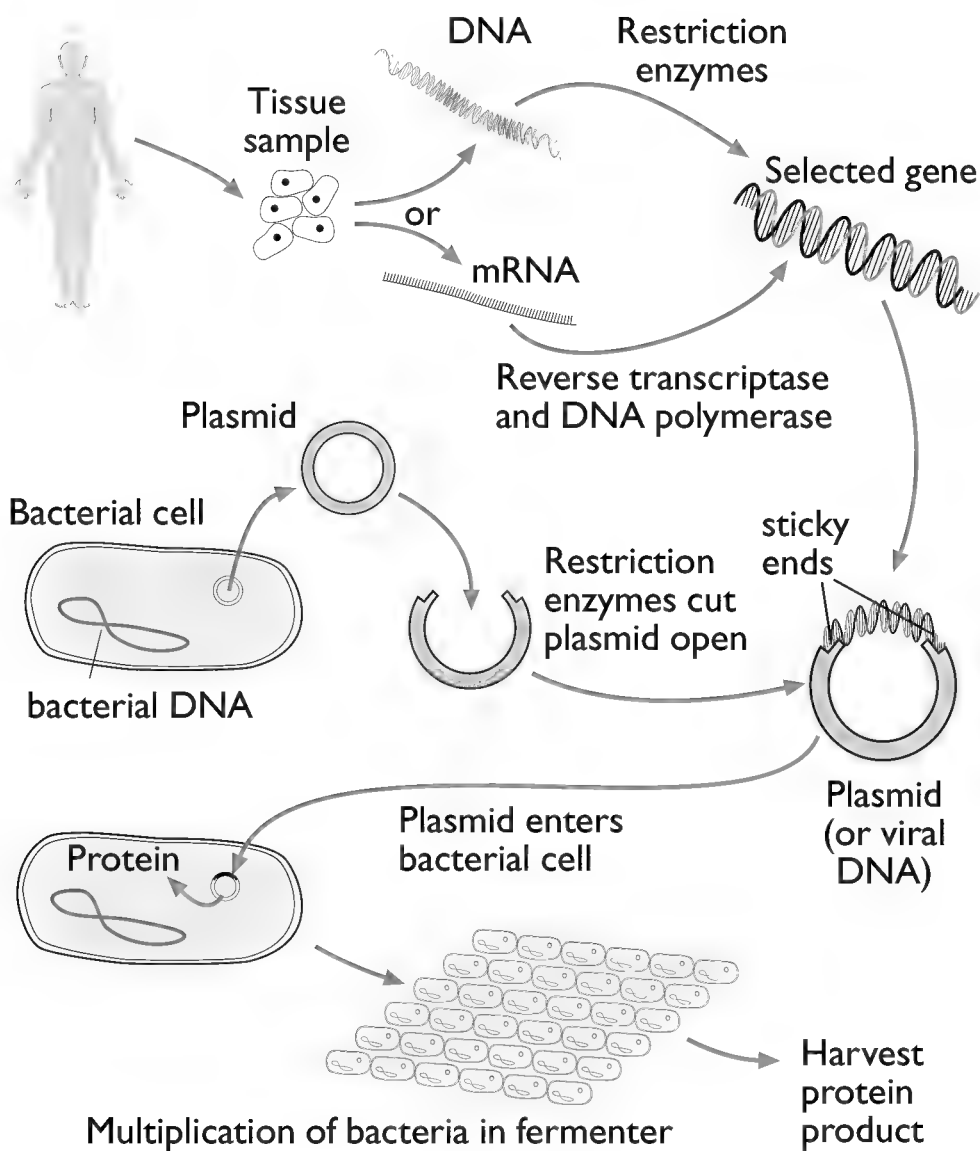
First meiotic division
Meiosis I



Second meiotic division
Meiosis II



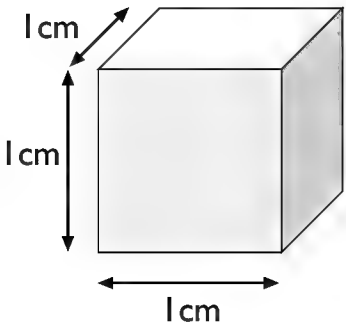
Haploid Gametes



Surface area = 6cm^2

Volume = 1cm^3

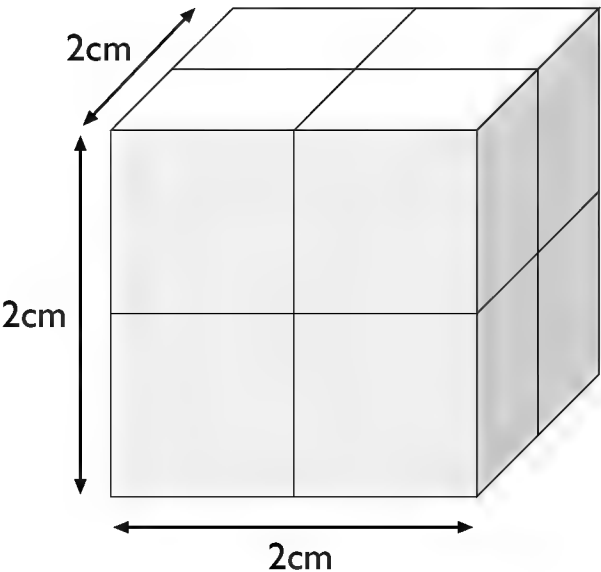
Surface area to volume = $6:1 = 6$

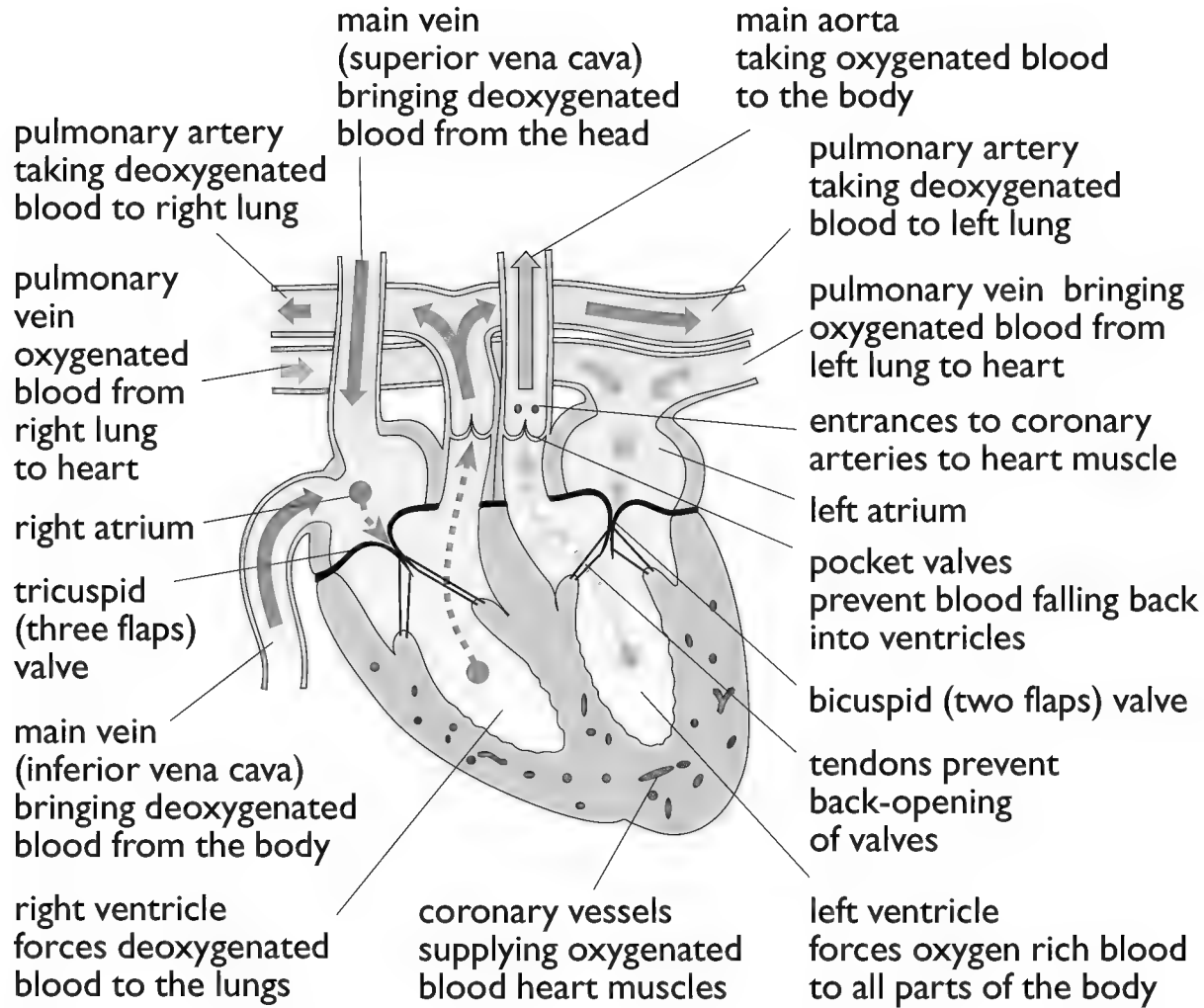


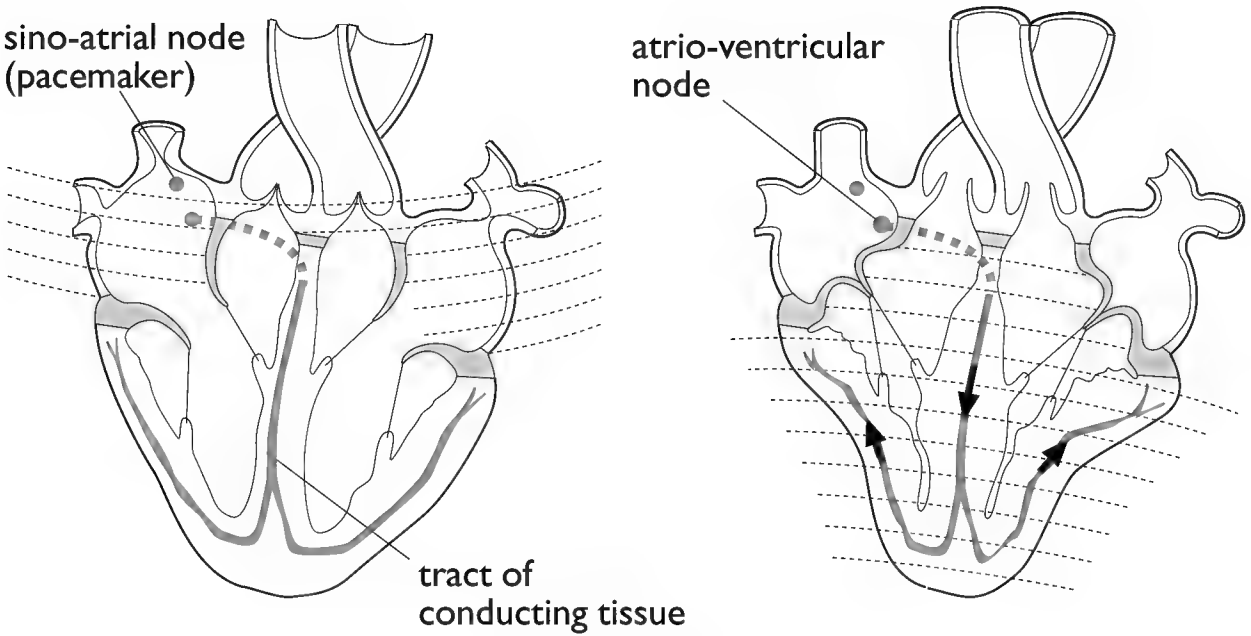
Surface area = 24cm^2

Volume = 8cm^3

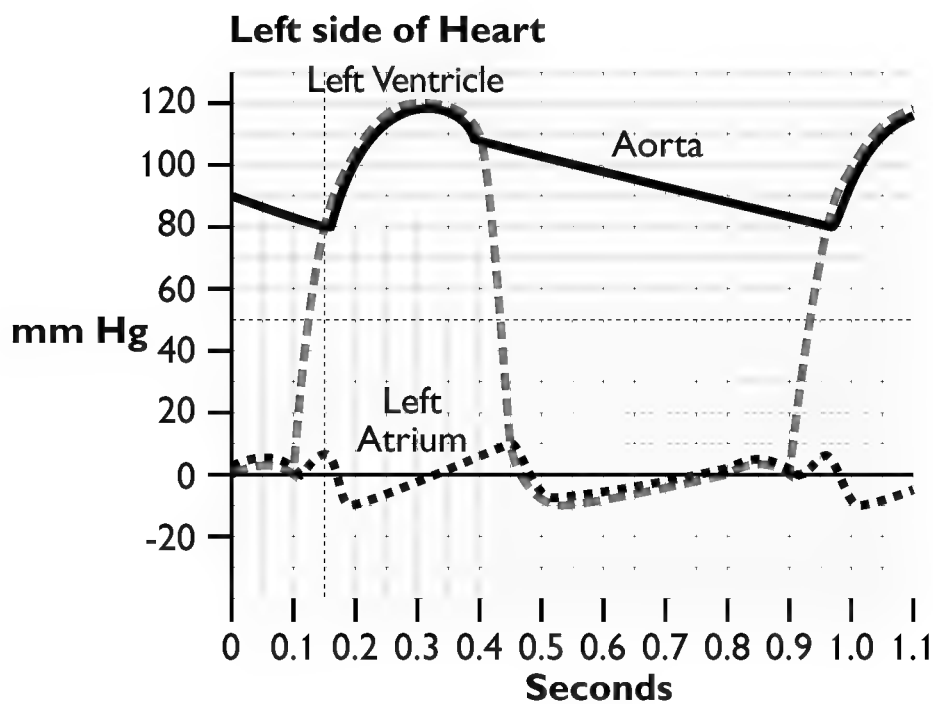
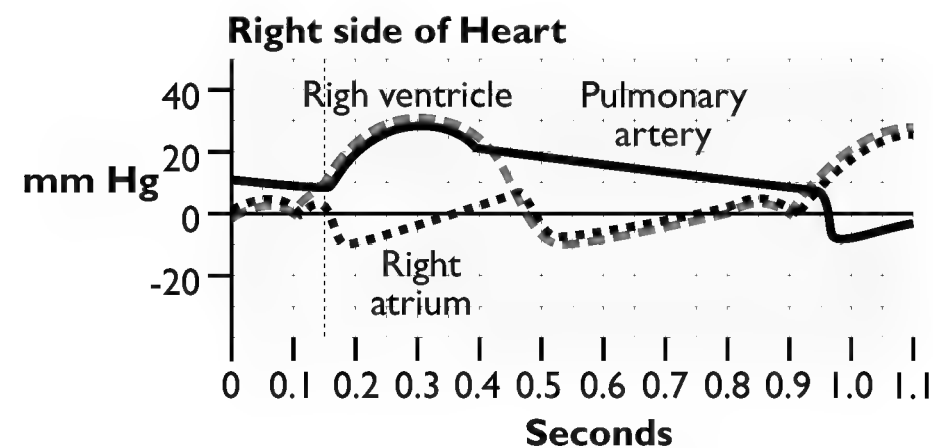
Surface area to volume = $24:8 = 3$

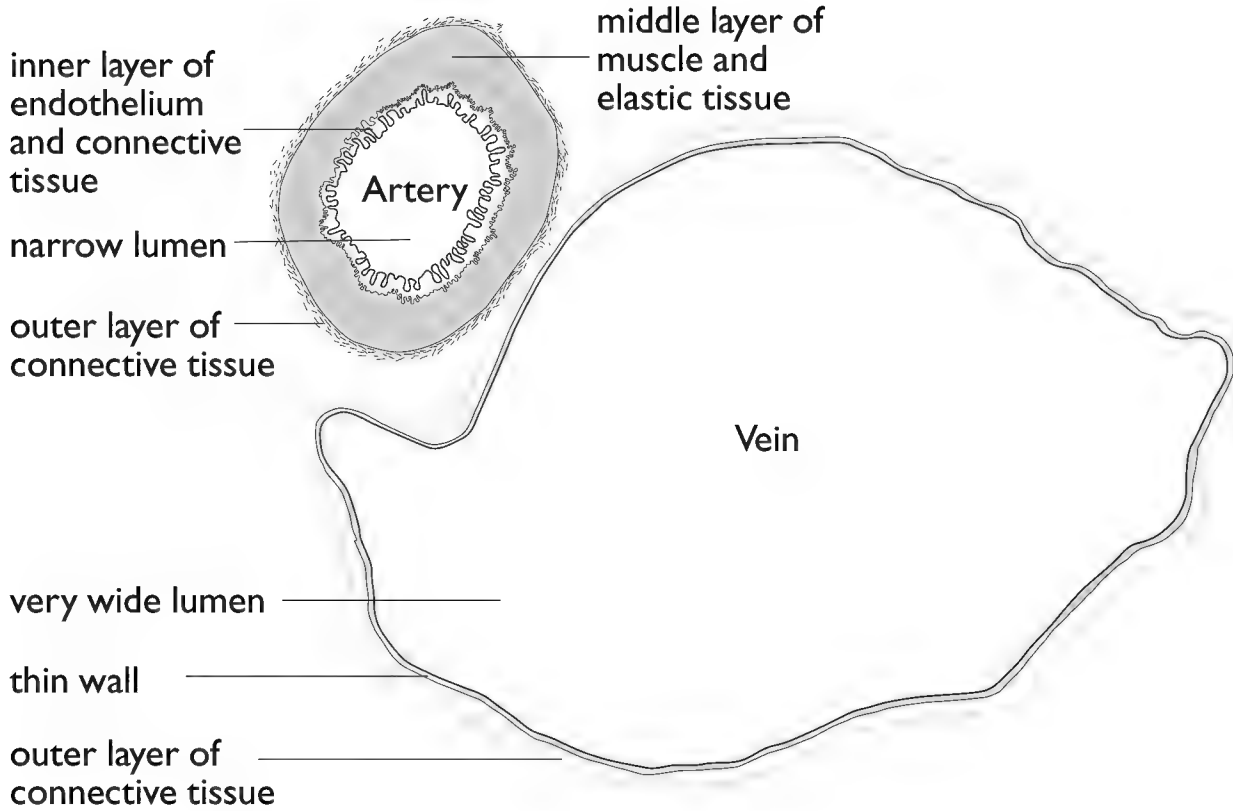




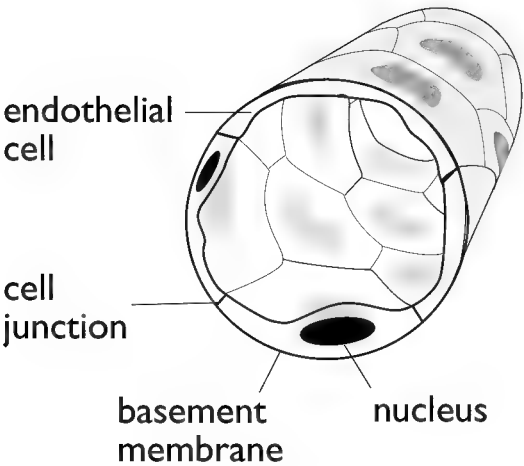


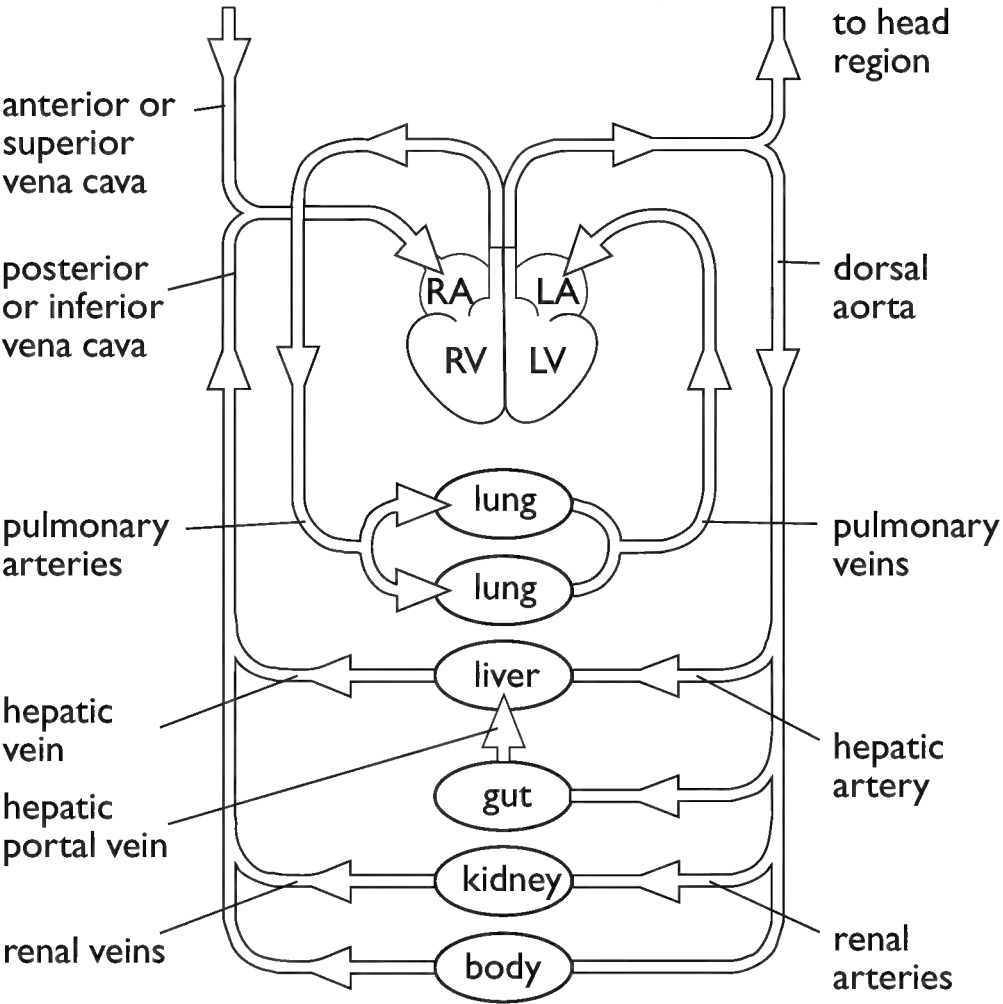
Graph - Chart of Pressure Changes During the Cardiac Cycle

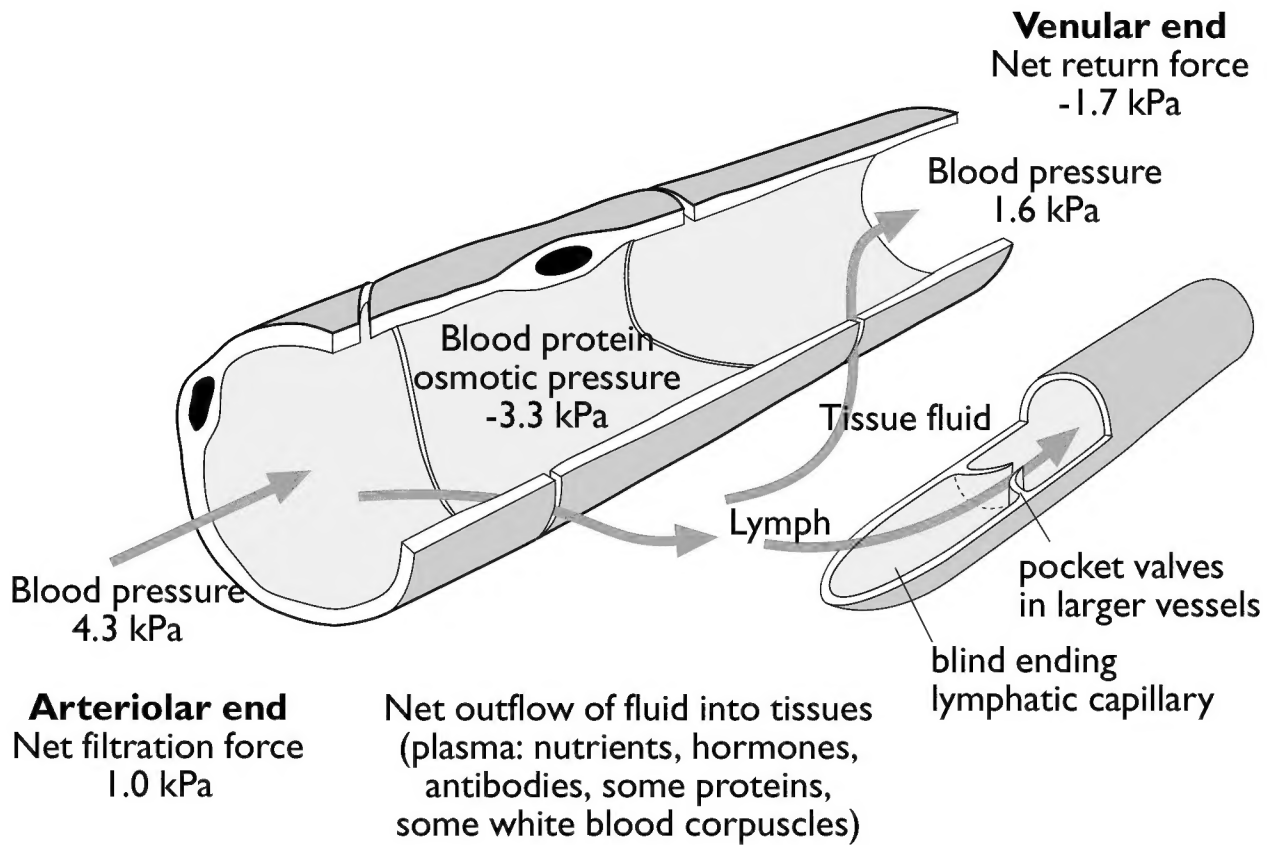


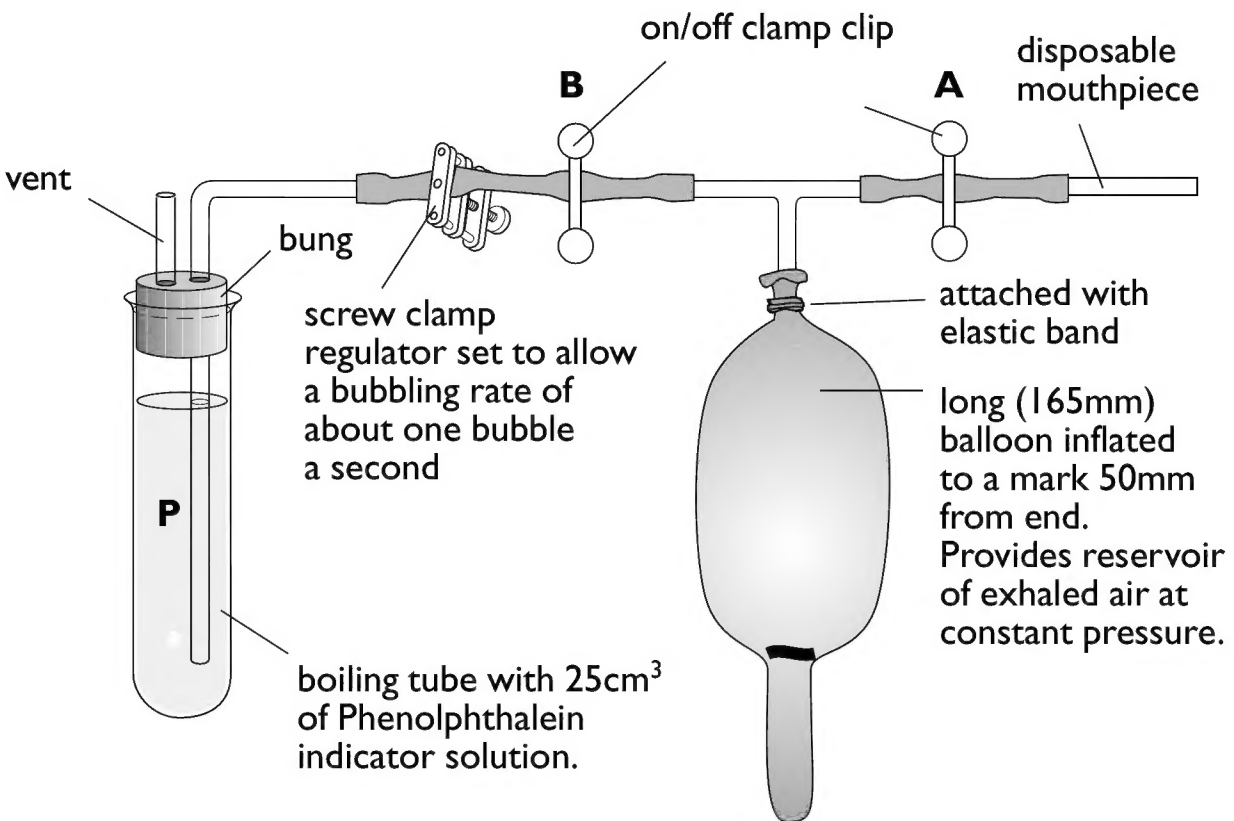


Typical capillary





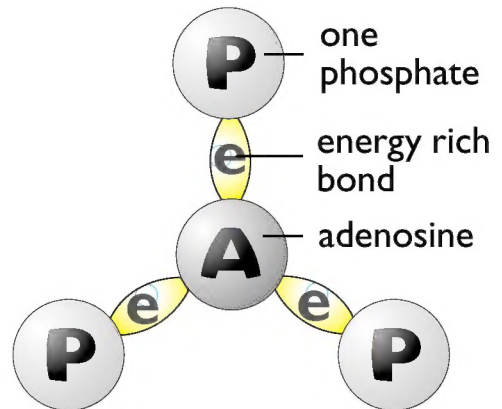




The body can only use energy in the form of **ATP**

Adenosine **T**ri **P**hosphate has 3 phosphate groups

If a phosphate group is removed from **ATP**, **energy** is released and **ADP** is formed



ADP has 2 phosphate groups

